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*GENETIC LINKAGE OF BLOOD TYPES IN THE RABBIT**

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Probably no human characteristics have been subjected, from a genetic standpoint, to more continuous and wide-spread observation, than have blood differences, the inheritance of which is accepted as being typically Mendelian. Yet association in inheritance of a human blood group or serological character with a morphological character has not been demonstrated, except in one doubtful case.[5]

With animals other than man the situation up to the present time has been similar. We are fortunate, therefore, in being able to report a first clearly demonstrated case of genetic linkage in the rabbit between a serological character, the so-called *A* character (presence of the *A* antigen *An* vs. the α agglutinin, *an*),[11, 13,14] and the morphological character brachydactyly (*br*).

We are also able to make a progress report on a study as yet incomplete in which genetic linkage is shown *not* to exist between the serological H_6 antigen and nine different and independent morphological characters. It remains to investigate the linkage relation of H_6 to two other morphological characters, satin (*sa*) and ataxia (*ax*).

The H_6 antigen is one of several antigenic differences known in the rabbit. The serological properties of the H_1 and H_2 antigens have been described by several authors and the inheritance of H_1 and H_2 has been studied and described in a series of publications by Castle and Keeler[2, 3, 7] who found H_1 and H_2 to be alleles and genetically independent of five known genes, viz., absence of the agouti pattern (*a*), dilution of coat color (*d*), non-extension of black pigment (*e*), the dominant white spotting (English) gene (*En*) and the short-haired (rex) gene (r_1). H_1 and H_2 were also found by Knopfmacher[8] to be independent of the following six genes: *a*, *an*, *ax*, *c*, *e* and *sa*. See Knopfmacher's paper for details and page 220 of this paper for significance of gene symbols.

The identity of the H_6 antigen as serologically distinct from and genetically independent of H_1 and H_2 was demonstrated by Knopfmacher[9]. In

extension of his work linkage tests have now been made which show the independence of H_6 from the mutant genes *a*, *br*, *c*, *d*, *e*, *En*, r_2, r_3 and *v*. See table 1. Study has not yet been made of the relation of H_6 to genes *sa* and *ax*, so it is still possible that a linkage may exist between H_6 and one of these genes.

TABLE 1

LINKAGE TESTS OF THE H_6 ANTIGEN WITH OTHER KNOWN GENES OF THE RABBIT

GENE	NUMBER OF PROGENY	NON-CROSSOVER	CROSS-OVER	DEVIATION	P.E.	DEV./P.E.
a	37	19	18	0.5	2.05	0.243
c	42	20	22	1.0	2.19	0.456
e	72	36	36	0.0	..	...
d	45	26	19	3.5	2.26	1.558
r_2	95	40	55	7.5	3.29	2.279
En	49	26	23	1.5	2.36	0.635
v	104	47	57	5.0	3.44	1.395
br	118	66	52	7.0	3.66	1.912
r_3	92	44	48	2.0	3.23	0.619

The usual method of linkage analysis, backcross of the double heterozygote to the double recessive, has been employed in these tests using the coupling heterozygous combination (*AB/ab*) in all cases except in the test involving English spotting. In this case English being a dominant mutation, the repulsion combination is more easily employed. Antiserums for the identification of blood types have been produced by the general method described by Knopfmacher,[9] with the exception that the injections made on successive days were progressively increased by 0.5 ml. until the last injection of 4 ml. was made. The animal was then rested five days and on the sixth day given a final dose of 4 ml., and bled five to seven days later. The antiserums used had a titer of at least 640. The H_1 antiserum had a titer of 1280. In typing animals the procedure described by Knopfmacher was routinely used and tests were made usually at birth for H_1, H_2 and H_6 at the same time.

Some irregularities such as were encountered previously by Castle and Keeler[2] and Battey[1] have also been observed in this study. Animals previously typed as negatives (lacking H_2 and H_6 antigens) during the summer months, when retyped during the cooler months of the year were found to possess these antigens. Some evidence, that such fluctuations are actually influenced by variations in temperature, has been obtained by Griffith[6] in a study of twelve animals possessing H_2 and H_6 antigens at normal room temperatures. When subjected over a period of two weeks, to temperatures ranging from 89–108°F., a definite drop in the strength of the antigen was observed. In some cases it disappeared entirely, but, in all cases, rose to the original titer with the return of the animal to normal room temperatures. To avoid uncertainty the results of the linkage tests

of H_6 with other known genes shown in table 1 include only observations made during the period from September 15 to June 15, when room temperatures are regularly lower than those which resulted in diminished titers.

Tests for the first two genes (*a* and *c*) listed in table 1 were completed but not published by Knopfmacher. The others have been made by us, but all alike show no statistically significant departure from the equality of crossover and non-crossover classes expected in independent assortment.

In studying the *A* character (*an*), both agglutination and double complement fixation tests were made for each animal. Since α agglutinins are not present until rabbits are about three months old, neither test was made prior to that age. In cases of uncertainty or disagreement between

TABLE 2

LINKAGE TESTS OF THE *A* CHARACTER (*an*) WITH SATIN (*sa*), VIENNA WHITE (*v*) AND BRACHYDACTYL (*br*)

GENE	TYPE OF MATING	NUMBER OF PROGENY	NON-CROSSOVER	CROSSOVER	DEVIATION	P.E.	DEV./P.E.	PER CENT CROSSING OVER
sa	Coupling	69	33	35	1.0	2.78	0.359	.....
v	Coupling	106	58	48	5.0	3.47	1.440	.....
br	Coupling	82	49	33	8.0	3.05	2.622	40.24 ± 3.72
br	Repulsion	32	23	9	7.0	1.91	3.644	28.12 ± 5.96
br	Total (*br*)	114	72	42	15.0	3.60	4.166	36.84 ± 3.16

the results of agglutination and inhibition tests, retests were made, the animal usually being bled a few weeks later.

Adequate tests for association between the *A* character and the mutant genes *a*, *c*, *d* and *En* were made by Sleeper[12] with negative results. Tests subsequently made by us for association between the *A* character (*an*) and the mutant genes *sa*, *v* and *br* are summarized in table 2. In the tests with *sa* and *v*, no evidence was found of linkage. But in relation to the *br* gene, evidence of linkage was found both in the coupling and in the repulsion matings. Of the 82 progeny obtained in the coupling matings, 33 (or 40.2 ± 3.7 per cent) were crossovers. In the repulsion matings 9 of the 32 progeny were crossovers, or 28.1 ± 6.0 per cent. Combining the two experiments gives us a population of 114 backcross individuals, in which 42 are crossovers, or 36.8 ± 3.2 per cent, a deviation from equality (expected if assortment is free) more than four times the probable error and so highly significant. This estimate is based entirely on segregation occurring among the gametes of F_1 females, in which the crossover percentage is regularly higher than in males, as has been demonstrated for rabbits, rats and mice, and is probably true for mammals in general.

It was shown by Castle and Sawin[4] that brachydactyl (*br*) is linked with furless (*f*) with a crossover percentage of about 28.3. Since this is considerably less than the estimated 36.8 per cent of crossovers between

br and the *A* character (*an*), it seems probable that the order of the three genes is *br–f–an* rather than *f–br–an*. The former gives a total map distance of less than 40, whereas the latter would make it in excess of 65, an improbably high figure.

Summary.—It has long been known that serological differences, such as the blood groups of man and other mammals, are inherited in typical Mendelian fashion and so are undoubtedly gene controlled. The first clear evidence of linkage between a serological gene and a gene for a morphological character, brachydactyl, is presented herewith. A third gene, furless, lies in the same linkage system with these two in what has been called the fifth chromosome of the rabbit.

Bringing up to date our knowledge of linkage in the rabbit, the six demonstrated linkage systems may be diagrammed thus:

Chromosome				Chromosome			
	c	*y*	*b*	IV	*a*	*dw*	*w*
I	0	14.4	42.8		0	14.7	29.9
	du	*En*	*l*		*br*	*f*	*an*
II	0	1.2	14.3	V	0	28.3	36.8
	r_1	r_2			*E*		*At*
III	0	17.2		VI	0		26.2

GENE SYMBOLS

a,	non-agouti	*dw*,	dwarf
an,	antigen *A*	*e*,	non-extension (yellow)
at,	atropinesterase[10]	*En*,	English spotting
ax,	ataxia	*f*,	furless
b,	brown (chocolate)	r_1,	rex No. 1, short hair
br,	brachydactyl	r_2,	rex No. 2, short hair
c,	albino	r_3,	rex No. 3, short hair
d,	dilute (blue)	*w*,	wide band
du,	Dutch spotting	*y*,	yellow fat

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[2] Castle, W. E., and Keeler, C. E., *Proc. Nat. Acad. Sci.*, **19**, 92 (1933).
[3] Castle, W. E., and Keeler, C. E., *Ibid.*, **19**, 98 (1933).
[4] Castle, W. E., and Sawin, P. B., *Ibid.*, **27**, 519 (1941).
[5] Finney, D. J., *Ann. Eugenics*, **10**, 171 (1940).
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[8] Knopfmacher, H. P., Thesis, Brown University (1942).
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[12] Sleeper, H. A., Thesis, Brown University (1940).
[13] Stuart, C. A., Sawin, P. B., Wheeler, K. M., and Battey, S. O. M., *J. Immunol.*, **31**, 31 (1936).
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CONCERNING LINKAGE OF WALTZING IN RATS

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Some mistakes in recent papers[1] on linkage in rats should be pointed out not only for the sake of accuracy in rat genetics but because the errors are of types made all too frequently in other fields. The mistakes stem from experiments that approach the problem repeatedly from one angle, which may result in an incompletely supported conclusion. They also include correct verbal logic inaccurately related to the experimental events and misapplication of the formula for computing the probable error to data which had been adjusted because of experimental necessity.

The idea that the recessive factors waltzing and albinism are linked seems to be a premature conclusion from experiments revealing only one side of the picture. In all the crosses reported in both papers the two genes were introduced into the F_1 through the same parent and were found together oftener than not in the F_2 and in testcross offspring. A variety of experiments of this coupling type, involving over 1000 rats, gave consistent results; yet there were no experiments to show whether the two factors, if introduced into the F_1 from *opposite* parents or in repulsion phase, would tend to remain *separated* in the gametes of these heterozygotes. Without such information from repulsion matings it is not evident how one can distinguish linkage of the two genes in inheritance from embryological enhancement of the waltzing in the albino phenotype. The latter is more than a remote hypothesis, because waltzers overlap with wild and are recognizable much less frequently than expected on the single factor hypothesis.

A second mistake, while made apart from the first, contributes further to the confusion between events in inheritance and in development. It is known that the recessive pink-eyed yellow gene is located in the same chromosome *pair* as the albino allels. Consequently, King and Castle say (p. 57), "If waltzing is linked with albinism, it should show linkage also with yellow." They performed a three-point experiment in which, it should be carefully noted, waltzing and albinism were introduced into

the F_1 from one parent while yellow was inherited from the other. Assuming linkage, one would expect from such a heterozygote an excess of those gametes containing either one or the other of the original combinations: waltzing, albinism, non-yellow or else non-waltzing, colored, yellow. In other words the waltzer and albino genes would most of the time remain coupled with each other and repulsed from yellow during meiosis. In

TABLE 1

OFFSPRING OF CASTLE AND KING'S TESTCROSS

$$F_1 \frac{C\ p\ W}{c\ P\ w} \text{ BY } \frac{c\ p\ w}{c\ p\ w}$$

GAMETES	CROSSOVERS IN REGION	OFFSPRING RECORDED (ADJUSTED NUMBERS IN PARENTHESES) GRAY, *Cc Pp*	YELLOW, *Cc Pp*	ALBINO, *cc ? p*	
C p W	0	.....	180 (129.6) normals	.....	
c P w	0	.....	.....	*S* 66 (145.2) waltzers in two *S* classes	
C P w	1	11 (24.2) waltzers	.....	.....	
c p W	1	.....	.....	*T* 175 (95.8) normals, in two *T* classes	
C p w	2	.....	42 (92.4) waltzers	.....	
c P W	2	.....	.....	*T*	
C P W	1 and 2	49 (35.8) normals	.....	.....	
c p w	1 and 2	.....	.....	S (see *c P w*)	
Totals		60	222	241	523

linkage inheritance certain genes tend to be held together in one chromosome while their allels are apt to be coupled in another group which is repulsed from the first. Forgetting that yellow would show repulsion from albinism and waltzer according to their hypothesis, the authors state (p. 58), "As expected, the percentage of waltzers in the yellow group is higher than that in the non-yellow, suggesting linkage of waltzing with yellow as well as with albinism."

Let us arrange the data from Castle and King's three-point testcross in a table to show (1) the occurrence of single, double and non-crossovers on the linkage hypothesis, and (2) the proportion of waltzers in each of the three coat-color classes. I am grateful to Dr. Castle for a suggestion which helped to simplify this table. Inspection of table 1 does not reveal the usual result of crossing-over, namely, a small number of double crossovers as compared with each of the single crossover classes. Since the color classes may be under suspicion of differentially enhancing waltzing, let us consider them separately. Among the gray rats there seem to be too many

double exchanges. Among the albinos too few non-crossovers appear. Among the yellows the percentage of crossovers between the *p* and *w* loci is low in comparison with the total data. In all cases the low classes are waltzer classes, which comprise only 22.8% plus or minus the standard error of 1.76%, whereas one would expect 50% waltzers if waltzing is differentiated from the normal by a single pair of genes. Consequently Castle has proposed a reasonable correction for this lack of waltzers.[2] He has added 1.2 times the waltzers obtained to each waltzer class at the expense of the corresponding non-waltzers. This gives the adjusted numbers shown in parentheses in table 1, where waltzers now equal non-waltzers in numbers. Other adjustments do not seem to be in order, since the 282 coloreds (grays plus yellows) are about equal to the 241 albinos; and the ratio of grays to yellows is an expression of the amount of crossing-over between those two loci, 22 units apart as shown here and previously.

TABLE 2

RECOMBINATION BETWEEN THE YELLOW AND WALTZER LOCI (I.E., ASSUMED CROSSOVERS IN REGION 2)

	%	S.E., %
Region 2 crossovers among grays (raw data)	81.7	± 4.96
Region 2 crossovers among grays (adjusted)	59.7	± 4.96
Region 2 crossovers among yellows (adjusted)	41.6	± 2.63
Region 2 crossovers among yellows (raw data)	18.9	± 2.63
Combined adjusted data	45.5	± 2.96
Probability of such a chance deviation from a 50% recombination, 0.13		

The effect of adjusting the data in the manner described is, among the coloreds, entirely upon the value obtained for region 2 (see table 2). In this table it will be noticed that the standard errors attached to the adjusted percentages of crossovers are the same as those computed for the actual samples. One could obtain a larger sampling error by treating the adjusted percentage as a raw sample, but I have not done so. The difference in the raw data is obviously great. The difference between the crossover values given by the gray classes and by the yellow classes, after the data have been adjusted in respect to waltzing, is still a very significant difference indicating that something more than linkage is operating. If the values of 59.7 and 41.6% crossovers had come from raw samples rather than from the adjusted figures nevertheless the difference between them could have occurred by chance alone only 1% of the time and should therefore be adjudged a real difference and one not to be merged by averaging. Considered another way, the 41.6% of yellows has a 0.016 probability of being a chance deviation from a 50:50 distribution; and the 59.7% of crossovers among grays could readily be a chance departure from independent assortment, *P* being 0.13, but in the opposite direction. Considered to-

gether the probabilities are thus better for independence than for linkage. Even if these differing measures of recombination are averaged, the result is on the borderline of a significant departure from independent assortment.

Using the raw data an important comparison is possible among those rats of this testcross which actually show waltzing. This comparison should be very meaningful because no adjustment, however reasonable, has been made for the expression of waltzing. All colored waltzers result from recombination, either in region 1 or in the provisional region 2. About 18% of each color are waltzers. The colored waltzers are distributed as follows: 11 gray waltzers, which would arise only from crossing-over in region 1; and 42 yellow waltzers, which result from an exchange in region 2. It follows from this that region 2 must be several times longer than the 22-unit distance contained in region 1. That would mean an exchange frequency great enough to represent free assortment between pink-eyed yellow and waltzing.

Since the correction for the poor expression of waltzing does not establish linkage to the satisfaction of this author, let us now see if any constructive conclusions using the contrary hypothesis of independent assortment may yet be drawn as to the penetrance of waltzing in different color classes. Among gray rats, which result from crossing-over between the *c* and *p* loci, waltzing is expressed in 18.3 ± 4.96% of the rats instead of the 50% expected without linkage. This gives a penetrance of 36.6% meaning that only 36.6% of the genetically waltzer rats showed that character phenotypically. Among yellow rats the proportion of waltzers is the same, 18.9 ± 2.63%. Assuming independent assortment of *w* and *y*, we would expect 50% waltzers, so the penetrance here is 37.8%. If linkage should be involved, the expected percentage would be below 50, and the resulting penetrance, for various assumed strengths of linkage, is shown in the right hand side of table 3.

In the remaining class, the albinos, the occurrence of waltzing is 27.5 ± 3.4%, higher than and perhaps significantly different from the frequency of waltzers among the grays and the yellows. The differences are twice the standard error of the respective differences, so that the odds are about 1 against 20 for them to be merely chance deviations. In the albinos the penetrance of waltzer turns out to be 55% if we assume no linkage. On the other hand, if we say that the penetrance is the same as in the colored classes, close linkage with only 10 or 20% of crossing-over would be indicated. No such close linkage has been found. Table 3 shows these and intermediate correspondences between crossing-over and enhancement of expression. The percentage of waltzers expected takes into account the merging of the double crossovers phenotypically into the non-crossover class as shown in table 1. It is assumed that double crossing-over takes

place without interference, so that the frequency of double crossovers equals the product of all exchanges for each of the two regions figured separately.

It may be true that both augmentation and linkage are responsible for this situation. In that case some other value of crossing-over, between the 35% tentatively arrived at by Castle and King (1941) and the free assortment suggested by myself, may turn out to be correct. A direct measure of penetrance could be obtained through data from inbred albino waltzer stock or from yellow waltzer stock independent of linkage complications. Separate measures of penetrance might through table 3 confirm some

TABLE 3

DEGREES OF PENETRANCE FOR THE WALTZER GENE, *w*, AS FIGURED FROM CASTLE'S TESTCROSS IF LINKAGE IS ACTING

ASSUMED % OF CROSSOVERS BETWEEN YELLOW AND WALTZER	% WALTZERS AMONG ALBINOS		PENETRANCE OF *w* IN ALBINOS, %	% WALTZERS AMONG YELLOWS		PENETRANCE OF *w* IN YELLOWS, %
	EXPECTED	FOUND		EXPECTED	FOUND	
10	72	27.5	38	10	18.9	..
20	67	27.5	41	20	18.9	95
30	62	27.5	44	30	18.9	63
40	56	27.5	49	40	18.9	47
45	53	27.5	51	45	18.9	42
Free assortment	50	27.5	55	50	18.9	38

particular value for crossing-over. Perhaps the difficulty in establishing the desired yellow waltzer stock is a low penetrance of waltzing in yellow rats. Within the limits of sampling errors the penetrances of waltzer in albinos and in yellows should lead to the same conclusion as to the frequency of recombination in the long and doubtful region. A more direct and unequivocal test would be an experiment starting with yellow waltzer mated to albino and testcrossing the F_1, but technical difficulties often slow one's realization of the conclusive demonstration.

A third type of mistake is a misapplication of a formula for computing the sampling error to data which do not comprise an actual sample. In the testcross transcribed in table 1 it was not possible to distinguish the albinos from the albino yellows, hence one of the two complementary crossover classes for region 1 could not be recognized. Sixty of the other class were found in a total of 523 offspring of all kinds. It was reasonable to double this 60 before dividing by the total in order to get the most likely per cent of recombination, visible and concealed. In figuring the reliability of this sample, the total of 523 seems to have been used giving a probable error of 1.2%. It would be more appropriate to use only the actually observed number of crossovers, 60 grays, and to divide that by the number of

non-albinos, or 282 coloreds. By thus removing the unclassifiable albinos a greater probable error is revealed, 1.6%. Even without this leeway the new crossover value for region 1, 21.3%, agrees better with the previous result of 21.9 ± 0.4% obtained by Castle and Wachter.

[1] King, Helen Dean, and Castle, W. E., these PROCEEDINGS, **23**, 56–60 (1937); Castle and King, *Ibid.*, **27**, 394–398 (1941).

[2] Castle and King, *Ibid.*, **27**, 396 (1941).

LINKAGE OF WALTZING IN THE RAT

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I am greatly indebted to Dr. Whittinghill for pointing out in his table 1 of the foregoing paper a serious mistake which I made in calculating crossover percentages in the data for the three-point cross involving waltzing, albinism and pink-eyed yellow (Castle and King, 1941). The mistake consisted in not making allowance for *double crossing-over*, i.e., crossing-over occurring simultaneously in regions *C P* and *P W* of the albino chromosome.

While the occurrence of linkage as originally assumed by Castle and King would still be shown, the map distances would be materially altered.

Let us then reëxamine the entire evidence in which linkage (coupling) between albinism and waltzing was postulated in 1937 and reasserted in 1941 with the additional conclusion that linkage in the repulsion phase was found between pink-eyed yellow and waltzing.

In the earlier (1937) paper cited, an attempt was made to summarize and interpret the results of three different crosses made between albino waltzers and colored non-waltzers known as hairless, kinky and blue, respectively.

All F_1 individuals were colored non-waltzers showing the recessive nature of both albinism and waltzing. In each of the F_2 populations four phenotypes were obtained, viz., colored non-waltzers (*C W*), albino non-waltzers (*c W*), colored wlatzers (*C w*), and albino waltzers (*c w*). In each of the F_2 populations fewer waltzers were obtained than were to be expected if waltzing is a simple recessive. This, however, is in accord with numerous earlier observations of King and is to be explained by the low effectiveness (penetrance) of the waltzing gene, so that many individuals which are homozygous for waltzing nevertheless do not waltz. The percentage of individuals which actually waltz is far below the expected 25% but higher among F_2 albinos (12%) than among F_2 colored individuals (5%), which

suggests the occurrence of linkage (coupling) between the recessive genes *c* and *w*, which entered the cross together.

To test this suggestion backcrosses were made between double recessive individuals (albino waltzers) and F_1 colored non-waltzers. From such matings 1:1 ratios were to be expected of colored and albino individuals, as well as of waltzers and non-waltzers, if both albinism and waltzing were single gene characters with normal complete penetrance. Substantial equality was realized between albino and colored classes, viz., *1318* albinos:*1365* colored. But again the incidence of waltzing was higher among the albinos (29%) than among the colored (22.7%), whereas 50% was to be expected in each case if penetrance were complete and linkage absent. Dr. Whittinghill has suggested that waltzing may be easier of realization in an albino than in a colored phenotype and that this rather than linkage may be the explanation of the difference in percentage of waltzers among albinos and among colored individuals. The suggestion is negatived by the result of a backcross in which a black waltzer (heterozygous for albinism) was mated with F_1 black individuals (cross 5 *C*, p. 58). Here the percentage of waltzers in the backcross population (20.9) was about the same as in other backcross populations, but a majority of the waltzers (43) were *black* while only 9 were albinos. This shows that waltzing is realizable in the usual percentage of cases in a colored as well as in an albino phenotype, and that the phenomenon under discussion is genuinely genetic, not merely developmental.

Dr. Whittinghill's further contention that a repulsion cross in addition to a coupling cross would be required for the complete demonstration of genetic linkage may be dismissed in the light of the repeatedly demonstrated fact that both in plants and in animals linkage in substantially like amounts will be shown by coupling and repulsion crosses involving in both cases the same pair of genes. The desirability of such supporting evidence may be freely admitted, but practical considerations at the time made it difficult to obtain.

We come now to a second line of evidence that linkage exists between albinism and waltzing, evidence which also is called in question by Dr. Whittinghill. Extensive experiments made many years ago showed that albinism (*c*) and pink-eyed yellow (*p*) are linked with about 22 per cent of crossing-over between them. Consequently, if waltzing is linked with albinism, it should show linkage also with yellow. The experiments described in our 1937 paper did not to our knowledge involve the yellow gene, until this fact was revealed by the appearance of the yellow phenotype in F_2 both in waltzing and in non-waltzing individuals. Then it became evident that the yellow gene had been introduced in our cross 3 as a recessive in the blue (colored, non-waltzing) parent. Among 63 F_2 yellows, 6 were waltzers. The number was too small to have statistical significance, but

in percentage frequency (9.5) was intermediate between that observed in other colored phenotypes (5.8) and that observed among the albinos (14.7). Dr. Whittinghill has quite correctly pointed out that "forgetting" the repulsion relationship in which the yellow gene had been introduced we hazarded an unsupported guess that waltzing might be linked with yellow as well as with albinism. Of course there was no statistically significant evidence for or against the suggestion and we did not assert that there was. But we did say (p. 58) that "Further experiments to test for linkage between waltzing and yellow [already in progress] are as yet incomplete and inconclusive." To obtain an adequate amount of data from such experiments required several years additional work. The results are contained in our 1941 paper.

A cross was made between pink-eyed yellow non-waltzers and albino waltzers. The F_1 animals were gray non-waltzers and their genetic constitution was reported thus, $\frac{C\ p\ W}{c\ P\ w}$. We were perfectly aware that this time p had been introduced in the repulsion relationship to waltzing for we had deliberately planned it that way.

After triple recessives (*cc pp ww*) had been laboriously synthesized, which in every gamete would transmit the three recessive allels, *c p w*, Dr. King backcrossed such triple recessives to F_1 mates obtaining a population of 523 animals distributed as follows:

	NORMAL	WALTZING	TOTAL	% WALTZERS	% CROSSOVER
Gray	49	11	60	18.3	18.8 ± 1.5 (*C/w* repulsion)
Yellow	180	42	222	18.9	
Albino	175	66	241	27.4	72.6 ± 1.8 (*c w* coupling)
Total	404	119	523	20.8	

That albinism is linked (coupled) with waltzing is indicated by the high percentage of albino waltzers (27.4). That the color gene (*C*) and the yellow gene (*p*) are *repelled* by waltzing is indicated by the much lower percentage of waltzers in the gray and the yellow classes (18.3 and 18.9, respectively).

Consider first the detailed evidence concerning the color gene (*C*) in relation to waltzing (*w*). All colored individuals would have derived *C* from their F_1 parent, which would produce gametes either *C W* (colored non-waltzers, gray or yellow) or *C w* (colored waltzers, gray or yellow). The progeny were 49 gray + 180 yellow = 229 non-waltzers, and 11 gray + 42 yellow = 53 waltzers, an indicated crossover percentage (repulsion between *C* and *w*) of 18.8 ± 1.5. From the albino group we may obtain additional evidence as to the linkage relations between *C* (or rather its recessive allele, *c*) and *w*. Gametes produced by the F_1 parent which would

give rise to albino offspring would be either *c W* (crossovers, non-waltzers) or *c w* (non-crossovers, waltzer). The numbers recorded are 175 crossovers to 66 non-crossovers, an indicated but highly improbable crossover percentage of 72.6. Such are the results based on the raw data the *p* locus being ignored, as it was in our 1937 experiments, in which the *p* gene was not involved except in the single experiment designated cross 3, as shown by the F_2 population of table 3, page 58.

Obviously it is necessary in dealing with a character having such low penetrance as waltzing manifests, to make a correction for the deficiency of phenotypic waltzers, as we did in our 1937 paper. This can be done by increasing the waltzer classes to their theoretical equality with half the entire population, and decreasing by like amounts the corresponding non-waltzer classes. In the present case, this may be accomplished by multiplying the recorded numbers of waltzers by the factor 2.2. The frequencies in each group then become as follows:

	NON-WALTZERS	WALTZERS	TOTAL	% WALTZERS	% CROSSOVER
Gray	35.8	24.2	60	40.3	41.3 ± 1.9 (*C/w* repulsion)
Yellow	129.6	92.4	222	41.6	
Albino	95.8	145.2	241	60.2	39.8 ± 2.1 (*c w* coupling)
Total	261.2	261.8	523	50.0	

Waltzers now, it will be observed, still appear as minority groups among the gray and the yellow classes, owing to repulsion between *C* and *w;* but among the albinos waltzers become a majority group, owing to coupling between *c* and *w*. The crossover percentage for repulsion in the combined gray and yellow classes (the entire colored population) is 41.3 ± 1.9, for coupling in the albino class it is 39.8 ± 2.1. For the entire population (colored and albino, repulsion and coupling experiments combined) it is 40.6 ± 1.4.

The value obtained for *C w* in our 1937 paper in which locus *p* was not involved was 44.7 ± 0.7 based on a population of 2139 backcross individuals. When a correction was made by increasing the number of waltzers to half the total population, the figure became 45.8 ± 0.7. It seems probable therefore that the true value lies between 40 and 45.

But if the *p* locus is taken into consideration, as it should be in a three-point cross, it is obvious, as Dr. Whittinghill points out that double crossing-over must be reckoned with, i.e., crossing-over simultaneously in region 1 (*C p*) and in region 2 (*p W*) which would result in the production of triple dominant gametes (*C P W*) potentially gray non-waltzers. These number 49 in the raw data and 35.8 in the corrected data.

Using first the raw data, the indicated crossovers in region 1 are 11 gray waltzers + 49 gray non-waltzers = 60 individuals or 21.3% of the colored

population. The indicated crossovers in region 2 are 42 yellow waltzers + 49 gray non-waltzers = 91 individuals, or 32.2 ± 1.8% of the colored population.

On this basis the linkage map for the *C p W* portion of the albino chromosome would be $\frac{C\ 21.3\ P\ 32.2\ W}{0 \qquad\qquad 53.5}$. The finding 21.3 for the *C P* region is in good agreement with the value 21.9 ± 0.4 found by Castle and Wachter (1924).

Let us now see how these values are affected by using the corrected data. The total amount of crossing-over indicated in region 1 (region *C p*) remains unchanged at 60 (24.2 gray waltzers + 35.8 gray non-waltzers) or again 21.3% of the colored population, which is in complete agreement with extensive earlier investigations. In region 2 (*p W*) the indicated crossovers are 92.4 yellow waltzers + 35.8 gray non-waltzers = 128.2 or 45.4 ± 1.97% of the colored population. Dev./P.E. = 2.3. A deviation from 50% of like magnitude might be expected by chance 12 times in 100, the odds against it being 7.28 to 1.

The linkage map would now become $\frac{C\ 21.3\ P\ 45.4\ W}{0 \qquad\qquad 66.7}$. The *C W* interval calculated without regard to *p* and based on the colored population only was 41.3 ± 1.9. When *p* is taken into consideration and double crossing-over allowed for, the interval becomes 53.5 based on the raw data, 66.7 based on the corrected data. Repulsion between *p* and *w* is 32.2 ± 1.8% based on the raw data, 45.4 ± 1.97% based on the corrected data. Calculated either way statistically significant coupling is shown between waltzing and albinism, and statistically significant repulsion between waltzing and the pink-eyed yellow gene is shown to be highly probable.

In the light of these findings the linkage map of the albino chromosome must be revised to show it considerably more extended than in our 1941 paper. It would appear about like this:

$$\frac{L\ C\ R\ P\ W}{0\ 3.3\ 3.8\ 25.2\ 70.6}.$$

Dr. Whittinghill's suggestion that it is desirable to base the probable error on the half-population under the circumstances described by him is entirely correct, as is also his insistance that double crossing-over and interference are not synonymous terms (p. 396). An error which he charitably calls "typographical" was due to an unfortunate aberration when I wrote non-curly instead of non-anemic in the second part of the 1941 paper (p. 398, line 9). I am obliged to him for pointing out these errors.

The slight differences between Dr. Whittinghill's figures and my own in certain instances are due to the fact that he uses standard errors and I probable errors.

X-RAY INHIBITION OF MITOSIS IN RELATION TO CHROMOSOME NUMBER*

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Numerous references to mitotic inhibition resulting from ionizing radiations appear in the literature, but the problem of the mechanism of this inhibition is still unsolved. Strangeways and Oakley,[1] Strangeways and Hopwood[2] and several subsequent investigators have shown that cells already in mitosis at the time of irradiation continue to divide, but that cells which normally would have entered mitosis during that period are inhibited at onset of prophase. Evidence that the inhibition is caused by the action of x-rays upon the nucleus was supplied by Henshaw and Francis (1936)[3] and by Henshaw (1938).[4] They found that x-rayed *Arbacia* sperm used to fertilize untreated eggs will delay cleavage, and Henshaw has shown that whereas irradiation of enucleated egg fragments has no effect on time of division irradiation of nucleated fragments caused delay in division. Crowther[5] suggested that the centrosome might be the structure involved in the inhibition, since the centrosome and the sensitive volume (calculated from the data of Strangeways and Oakley[1]) were of the same order of magnitude. Fröier, Gelin and Gustafsson[6] concluded that extra-chromosomal processes within the nucleus influence the onset of prophase and, when disorganized by x-rays, they prevent mitosis. This inference was based on the ability of cells to divide even when very high x-ray doses have broken up the chromosomes to the extent that they have lost all distinct continuity and shape. However, other interpretations will fit their observations. Previous investigation by one of us showed that there is no correlation between chromosome length and mitotic inhibition.[7] In this paper we wish to report evidence that inhibition of mitosis is dependent upon the number of chromosomes in the cell.

Root tips of three species of *Triticum* (*T. monococcum*, *T. dicoccum* and *T. vulgare*) and two species of *Bromus* (*B. carinatus* and *B. arizonicus*) were selected as the biological material. Both genera are members of the Gramineae and both have basic diploid chromosome numbers of 14. The numbers of chromosomes in these five species form a series of 14, 28, 42, 56 and 84. The source of x-rays was an oil-cooled tube with a tungsten target in a General Electric Company Maximar apparatus, operated at 220 Kvp. and 15 ma. The beam was filtered through 1 mm. Al, and a lead diaphragm 1 cm. thick and with a 10 × 10 cm. aperture was used at the base of the cone which was 17.5 cm. in length. The target distance was 85 cm. Irradiation was given at the rate of 43 r per minute as measured with a Victoreen ionization chamber.

Seeds were germinated on moist filter paper in covered glass dishes kept at a constant temperature of 25°C. When the roots were 2 to 3 cm. long, seedlings of each species were irradiated in five different lots with increased dosage for successive lots. After irradiation the seedlings were returned to the incubator for 3 hours. Cells at the onset of prophase when irradiated are in anaphase 3 hours later.[7] At the end of that interval root tips were fixed in a solution of 1 part 50% ethyl alcohol : 1 part 50% acetic

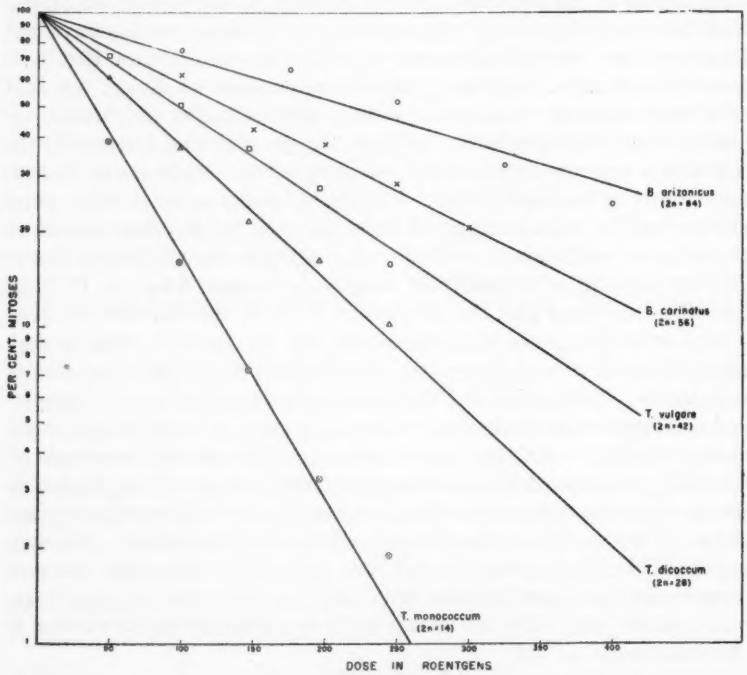

FIGURE 1

Logarithms of per cent mitoses as a function of dose in roentgen units.

acid. The fixation period was 18 to 20 hours, after which the root tips were left in 70% alcohol for approximately 24 hours. They were then washed in distilled water for 10 minutes, treated with 4% iron alum for 1 hour, washed in distilled water for 1 hour, and macerated in concentrated hydrochloric acid for $1^1/_2$ minutes. After washing them again in distilled water for 20 minutes or longer, aceto-carmine smears were made. Sufficient length of each root tip to insure the inclusion of the entire region of cell division was smeared. Two small drops of aceto-carmine were placed close together on the slide, in one of which two root tips were gently

mashed with a dissecting needle to separate the cells. Cells adhering to the needle were then washed off in the other drop of stain and a cover glass placed over both drops and their contents. To prevent the escape of any cells from under the cover glass, care was taken to use just enough aceto-carmine to extend to the edge of the cover glass when the smear was adequately flattened. Control root tips for each experiment received treatment identical with that given irradiated root tips, with the exception of exposure to x-rays.

All metaphases and anaphases were counted in 8 to 18 root tips of each control or irradiated lot. Prophases and telophases were not included in the counts because of difficulty in distinguishing accurately between late resting stage and early prophase and between late telophase and early resting stage. The maximum variation between mitotic counts for any two slides of one lot are as follows: *T. monococcum* 11%, *T. dicoccum* 34%, *T. vulgare* 40%, *B. carinatus* 25% and *B. arizonicus* 33%. The mean deviations for the same species were 8, 13, 20, 15 and 16%, respectively. Percentages of the average number of mitoses per control root tip were found for each irradiated lot. In figure 1 the logarithms of these percentages are plotted against dosage. The curves are all of the form $Y = e^{-kx}$, where Y = per cent mitoses, e = the base of natural logarithms, x = dose in roentgens and k = a constant. They were fitted by the method of least squares with each point weighted by the number of mitosis observed. In table 1 the slopes of the curves are given, together with the root mean square deviations and the measures of goodness of fit according to the equation

$$R^2 = 1 - \left[\frac{\Sigma(y-Y)^2}{n\left(\frac{\Sigma(y^2)}{n} - \frac{(\Sigma y)^2}{n}\right)} \right]$$

where y = observed log precentage mitoses, Y = the calculated log percentage mitoses and n = the number of points on the curve (Yule and Kendall).[8] Values of the slopes (k) and root mean square deviations (S) are given in terms of the base e of natural logarithms.

TABLE 1

GENUS AND SPECIES	CHROMOSOME NUMBER ($2n$)	$k_e \times 10^{-3}$	$S_e \times 10^{-3}$	R
Triticum monococcum	14	17.698	1.29	0.994
Triticum dicoccum	28	9.721	0.53	0.999
Triticum vulgare	42	6.997	0.46	0.999
Bromus carinatus	56	5.197	0.49	0.999
Bromus arizonicus	84	3.159	0.52	0.996

In order to verify previous evidence that inhibition of mitosis is not a function of chromosome length, chromosomes of each species were meas-

ured. Two types of measurements were made; in one, all chromosomes of a single metaphase cell from each species were measured from camera lucida projections, with the usual precautions taken to avoid optical distortion. Where a chromosome was not entirely horizontal, its length was determined geometrically from the highest and lowest focal points of those portions which deviated from the horizontal. Since this procedure is very laborious, additional measurements were made with the ocular micrometer. All chromosomes in 10 cells of *T. monococcum*, in 8 cells of *T. dicoccum*, in 5 of *T. vulgare*, in 4 of *B. carinatus* and in 2 cells of *B. arizonicus* were measured by the latter method. Camera lucida and ocular micrometer measurements are listed separately in table 2. Lengths are given in terms of total chromosome length, i.e., the sum of the lengths of all chromosomes in a metaphase configuration.

TABLE 2

TOTAL CHROMOSOME LENGTHS (IN MICRONS)

SPECIES	LENGTH (1 CELL) (CAMERA LUCIDA)	MEAN LENGTH (OCULAR MICROMETER)	RANGE OF LENGTHS (OCULAR MICROMETER)
T. monococcum	124	118 (10 cells)	114 to 127
T. dicoccum	219	203 (8 cells)	194 to 222
T. vulgare	243	248 (5 cells)	238 to 286
B. carinatus	332	287 (4 cells)	274 to 336
B. arizonicus	411	374 (2 cells)	358 to 387

In connection with these data on chromosome length, certain results of the investigations of Fröier, Gelin and Gustafsson[6] are of interest. They have reported the number of cells showing abnormalities following irradiation of dry seeds of the same *Triticum* species used in this work. They point out that there are fewer abnormalities in *T. vulgare* than expected from calculations based on the rate of production of abnormalities in *T. monococcum*. However, their calculations are based on the assumption that the mean length of the chromosomes is the same in the two species. Our measurements show that the total chromosome length in *T. vulgare* is not three times that of *T. monococcum* as assumed by those authors, but is only twice as great. Their observation is in agreement with evidence from a variety of other species that chromosome sensitivity is directly proportional to the total length of the chromonemata or roughly proportional to total chromosome length. Furthermore, from the combined evidence concerning chromosome abnormalities and chromosome lengths one can conclude that the chromosomes of *T. vulgare* are shorter than those of *T. monococcum* because their chromonemata are shorter in absolute length and are not merely more tightly coiled than those of *T. monococcum*.

In table 3 are given the ratios of the chromosome numbers of the different species, the inverse ratios of the slopes of their "survival" curves and the ratios of the total lengths of the chromosomes of their complements.

There is a linear proportionality between the ratios of the chromosome numbers and ratios of slopes, the greatest deviation being 18%. This relationship may be demonstrated in another way: when the slopes are multiplied by the multiples of 14 represented in their respective chromosome numbers, i.e., 1, 2, 3, 4, and 6, the resulting figures are 17.7, 19.4, 21.0, 20.8 and 19.0, or very nearly a constant. No such relationship exists between ratios of chromosome lengths and ratios of slopes.

TABLE 3

RATIOS OF CHROMOSOME NUMBERS	INVERSE RATIOS OF k	RATIOS OF CHROMOSOME LENGTHS (CAMERA LUCIDA)	RATIOS OF CHROMOSOME LENGTHS (OCULAR MICROMETER)
56/42 = 1.33	1.35	1.37	1.16
42/28 = 1.50	1.39	1.11	1.22
84/56 = 1.50	1.63	1.24	1.30
28/14 = 2.00	1.82	1.77	1.72
56/28 = 2.00	1.87	1.52	1.41
84/42 = 2.00	2.21	1.69	1.51
42/14 = 3.00	2.54	1.96	2.10
84/28 = 3.00	3.07	1.87	1.84
56/14 = 4.00	3.42	2.68	2.43
84/14 = 6.00	5.60	3.33	3.18

From these data it becomes evident that for the species studied mitotic survival is directly proportional to multiples of the genome, i.e., with every duplication of chromosome number the survival rate is doubled, or conversely, the mitotic inhibition is reduced by one-half. Since inhibition varies inversely as the chromosome number, but is not similarly related to chromosome length, structures within the chromosomes, either single or if multiple then not at all uniformly distributed along the chromosomes, must be involved in the mechanism of mitosis. Mitotic failure is then caused by inactivation of a minimum number of these structures. The only visible chromosomal structures known which fulfil these requirements are the centromeres (spindle-fibre attachment regions). These results might also be obtained if a small number of genes were involved, but not by a considerable number of genes distributed throughout the length of the chromosomes.

Of these two alternatives, the first is supported by a qualitative result obtained consistently with irradiated cells. Lagging chromosomes, groups of chromosomes or fragments of chromosomes form their own little nuclei when the major portion of the complement enters the telophase. These micronuclei retain their structural integrity until the major nucleus enters the prophase. At that time the micronucleus will go through the characteristic prophase-anaphase changes if its chromosomal components contain centromeres. If they do not, the micronucleus becomes pycnotic and then disintegrates.[9] The presence of the centromere thus appears to

be essential for the initiation of those changes associated with the onset of prophase.

If calculations of sensitive volumes are made from the above data, the result obtained is peculiar in that the sensitive volume decreases as the volume of chromosomes is increased. This contrasts strangely with the observations on the production of chromosome abnormalities with x-rays and neutrons, where the sensitivity is directly proportional to the length of the chromonemata and the diameter of the sensitive portion of the chromonema is constant.[7, 10] It differs also from the result obtained with virus and bacteriophage where the calculated sensitive volumes were found to be proportional to the size of the organism as determined by their ability to pass through filters of different pore size.[11, 12] Superficially the results obtained on inhibition of mitosis would appear to contradict the concept of sensitive volumes, but more careful consideration shows this not to be the case at all. If the initiation of the mitotic process requires the participation of a certain minimum number of structural units, e.g., 7 out of 14 centromeres or genes in the diploid, the same number 7 may be required in the polyploids and not one-half the total number. Analogous situations are common in the phenotypic expression of allelic genes where the presence of a single dominant factor suffices to bring about the same phenotypic expression as two or more such alleles. The radiation will reduce the number of active mitotic centers in proportion to the number present. Thus if one-half are inactivated by any given dose, this will leave 7 active in the diploid, 14 in the tetraploid, etc. The dose must therefore be increased in the tetraploid in order to reduce the number of active centers to the point where mitosis is inhibited. It will be clear therefore that this case cannot be analyzed in terms of sensitive volumes, where the basic assumption for the necessary calculations is that each functional unit contribute to the process under consideration.

It is of interest to note that the diameter of the sensitive volume 2.2×10^{-5} cm. for *T. monococcum*, which has the basic chromosome number, is close to that calculated by Crowther[5] for chick fibroblasts, i.e., somewhat less than 4×10^{-5} cm.

Further investigation of this problem is in progress.

Summary.—1. Inhibition of mitosis by x-rays is inversely proportional to the number of chromosomes in the species studied.

2. Inhibition of mitosis is neither directly nor inversely proportional to chromosome length.

3. The evidence indicates that inhibition of mitosis by x-rays occurs through action upon the centromeres.

4. The significance of these findings in connection with sensitive volume calculations is discussed.

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EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA I. GEOGRAPHIC STRAINS OF DROSOPHILA WILLISTONI

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If mature and sexually active females and males of two or more animal species inhabit the same portion of space they do not mate at random. For various reasons females of each species mate with and are inseminated by males of their own species more often than by males of other species. Causes which make mating and insemination selective rather than random may be grouped under the categories of sexual and mechanical isolation. Sexual (psychological) isolation includes various differences in behavior, in smells, and in adornments which make females and males of different species less likely to find each other, and if found less attractive and therefore less willing to accept the courtship and invitation to mating. Mechanical isolation refers to differences in the structure or function of the genitalia and other body parts which play a rôle in the act of copulation and which handicap normal intromission and sperm delivery. The distinction between sexual and mechanical isolations is to a certain extent arbitrary, and it is probable that with more study situations will be found which will be intermediate or will require a different classification.

Aside from sexual and mechanical isolation, there are other causes intrinsic to the organisms concerned which handicap or prevent the gene exchange between populations of different species. We have proposed to designate all such causes reproductive isolating mechanisms, and have reviewed them elsewhere.[1-4] Gene exchange between pairs of species is prevented usually not by a single but by several isolating mechanisms; each mechanism by itself is frequently inadequate to maintain the species separation but they do so when combined. Furthermore, the separation of different pairs of species, even within the same genus, is often caused by different sets of isolating mechanisms. It must be emphasized in this connection that a complete isolation of species is not necessarily accompanied by hybrid sterility. Unquestionably good species, including such that are classified in different subgenera or genera, are sometimes not separated by sterility barriers. The Mallard (*Anas platyrhynchos*) and the Pintail (*"Dafila" acuta*), the two most common ducks of the temperate zone of the northern hemisphere, coexist without mixing over many millions of square miles even though the F_1, F_2, and backcross hybrids are completely fertile (Phillips[5]). Only a few natural hybrids between these species have been found (less than one in 50,000 killed). Gordon[6] found a high fertility of hybrids but absence of interbreeding in nature between

sympatric species of the fish genera *Platypoecilus* and *Xiphophorus*. We believe that sexual isolation is the most important of isolating factors in many groups of animals (mammals, birds, most insects). This is not because sexual isolation prevents the gene exchange between species any more securely than do other isolating mechanisms, such as, for example, inviability of hybrids and hybrid sterility. The advantage of sexual isolation is on a different plane: it permits two or more species to occupy the same general territory without a reproductive wastage of producing poorly viable or sterile individuals. This consideration suggests that natural selection might favor the establishment of sexual isolation as early as possible during the process of speciation.

One of the first questions that arises when we consider sexual isolation or any other isolating mechanism is whether it occurs only between species or, in at least rudimentary form, between geographic races as well. The answer to this question may clear up the problem of the origin of isolating mechanisms. If the theory is valid that species emerge gradually from geographic races then indications of incipient isolation should occasionally be encountered between geographic races, exactly as all other differences between species are foreshadowed in this manner. Dobzhansky and Koller[7] found that the races of *Drosophila miranda* which inhabit the state of Washington and the Sierra Nevada of California respectively show a weak preference for homogamic mating, and the same has been found by Stalker[8] to be true with respect to strains of *D. americana* coming from different localities in the state of Ohio. Matings among members of the same strain, race, or species shall be referred to as homogamic and those between different strains, races, or species as heterogamic. The method used by these authors for investigating sexual isolation is the so-called multiple choice technique: one kind of males is placed in a container with two kinds of suitably marked females, and it is tested whether and to what extent the insemination is at random. This is determined by dissection of the females after a certain amount of time has elapsed and by examination of their seminal receptacles to see whether or not they contain sperm. Provided that only a part of the females have been inseminated, it is easy to tell whether the insemination of the two kinds of females takes place selectively or at random. The method has the drawback that it gives a joint estimate of the magnitude of sexual and of mechanical isolations. Theoretically, the same frequency of insemination may occur because males of the strain A do not attempt to copulate with females of B, or because the copulation is not completed on account of an incongruence of the genitalia. Although mechanical difficulties for copulation are probably not encountered in crossing races or closely related species of Drosophila, the method gives no information about which particular link in the chain of processes leading from courtship through copulation to insemination may

be responsible for the selective insemination obtained. Finally, although it is easy to determine whether or not a female has some sperm in its seminal receptacle or its spermathecae, it is difficult to estimate the quantity of the sperm. It is possible that in some copulations the amount of the sperm delivered is less than in others. Patterson, Stone, and Griffen in *D. virilis* and *D. americana*, Stone in *D. hydei*, Griffen in species of the *D. melanica*, and Mainland in those of the *D. funebris* group recorded the proportions of pair matings which produce offspring under uniform laboratory conditions.[9] They found that in some instances this proportion is greater when the female and the male mated are derived from the same than when they come from different geographic strains. It is probable that this is due in part to incipient sexual isolation between geographic races of the same species, although the production of offspring evidently depends on many more variables than insemination does.

D. willistoni Sturtevant is a species common in tropical America; its known distribution extends from the southern tip of Florida to the southern part of the state of São Paulo, i.e., from just north of the Tropic of Cancer to just south of the Tropic of Capricorn. We have living strains which fit the description of *D. willistoni* from Praia Grande and Bertioga (state of São Paulo), from Rio de Janeiro, Belem (state of Pará), Teffé (state of Amazons) in Brazil, and from Quiriguá in Guatemala (Fig. 1). With the possible exception of Quiriguá, each of these strains is derived from several females caught in the respective localities; the Quiriguá strain has been obtained through the courtesy of Professor A. H. Sturtevant. The Teffé strain fails to cross, except very rarely, with any of the other. This situation will be dealt with in another communication, and here we are concerned with the other five strains.

The experiments were arranged as follows. Ten virgin females of a given strain are placed in a $3^3/_4 \times 1$-inch vial, in which a small amount of the cornmeal-molasses-agar medium was poured and allowed to solidify in a slanting position; ten virgins[10] of another strain and ten freshly hatched males of one of these strains are added. Since the flies from the different strains are not distinguishable in appearance, the right wings are clipped off in one and the left wings in the other class of females. The vials are put in an incubator at $24^1/_2$°C. for 40–55 hours, whereupon all the females are dissected and their tubular receptacles and spermathecae examined under a microscope for sperm. Fertilized females have spermatozoa as a rule both in the receptacle and in the spermathecae, only a few individuals having been found showing sperm in the receptacle only. For statistical purposes it is best to have approximately one-half of all females inseminated at the time of the dissection; this time was adjusted accordingly, but some dissections have also been made of females which were exposed to the males for 24 and 72 hours. The data obtained in 24-

and 72-hour dissections serve as a check on the possibility that there is a significant excess of homogamic or heterogamic matings among the first or the last flies fertilized in a culture. This possibility is not realized.

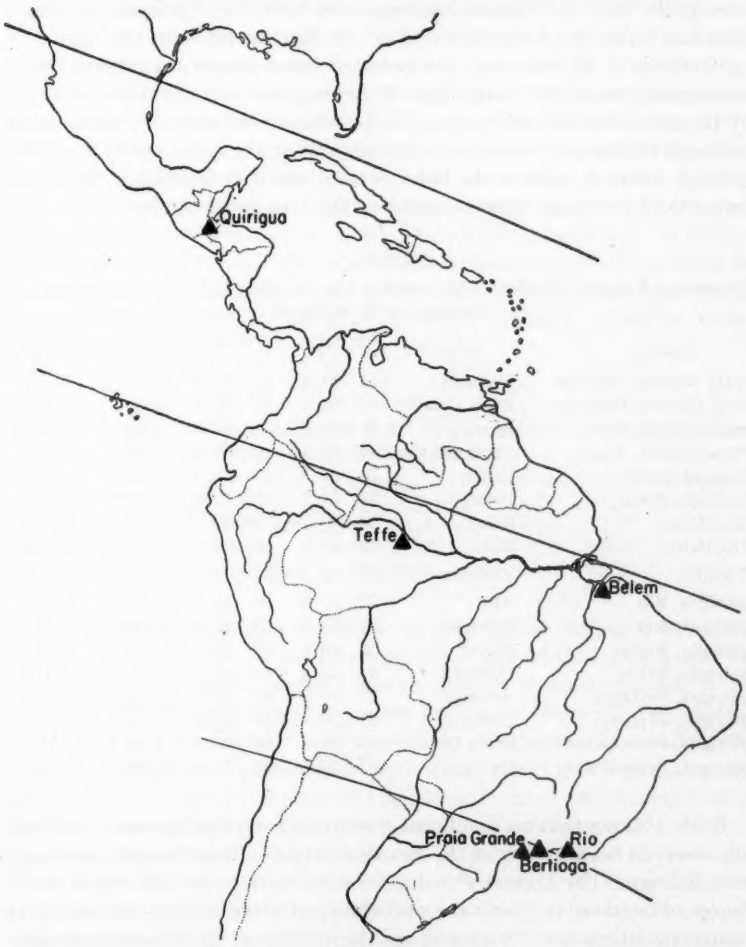

FIGURE 1

A map showing the localities in which the strains of *D. willistoni* mentioned in the text have been collected.

A summary of the data is presented in table 1 (except that the 24- and 72-hour dissections and the occasional other cultures in which too few or too many females were fertilized are not included). In this table the per-

centages of homogamically and heterogamically fertilized females are given for every cross, together with the χ^2's which measure the statistical significance of the differences observed between the frequencies of the homogamic and heterogamic matings, and with the "isolation indices." This last value has been proposed by Stalker[8] to measure the degree of randomness of the matings; the isolation index equals the percentage of homogamic minus the percentage of heterogamic inseminations, divided by the sum of these percentages. If the isolation between the forms tested is complete and only homogamic matings occur the index is +1.0; if the matings occur at random the index is zero, and it is negative if there is a preference for matings between representatives of different strains.

TABLE 1

NUMBER OF FEMALES DISSECTED (*n*) AND PER CENT CARRYING SPERM (%) IN VARIOUS CROSSES OF *D. willistoni*

FEMALES	MALES	HOMOGAMIC		HETEROGAMIC		χ^2	ISOLATION INDEX
		n	%	*n*	%		
Praia Grande, Bertioga	Bertioga	57	54.4	57	57.9	0.10	−0.03
Praia Grande, Bertioga	Praia Grande	74	66.2	73	65.8	0.003	0.00
Praia Grande, Rio	Rio	69	60.9	71	69.0	1.02	−0.06
Praia Grande, Rio	Praia Grande	59	66.1	64	56.2	1.29	0.08
Bertioga, Belem	Belem	73	60.3	78	56.4	0.16	0.03
Bertioga, Belem	Bertioga	73	71.2	78	52.5	5.49	0.15
Rio, Belem	Belem	69	65.2	71	62.0	0.16	0.03
Rio, Belem	Rio	64	50.0	60	58.3	0.88	−0.08
Brazilian	Brazilian	538	62.1	552	59.8	0.61	0.02
Quiriguá, Rio	Rio	57	57.9	60	40.0	3.70	0.18
Quiriguá, Rio	Quiriguá	59	39.0	63	41.3	0.06	−0.03
Quiriguá, Belem	Belem	68	48.5	74	25.7	7.97	0.31
Quiriguá, Belem	Quiriguá	64	45.3	60	48.3	0.13	−0.03
Quiriguá, Bertioga	Bertioga	77	66.2	69	20.3	31.02	0.53
Quiriguá, Bertioga	Quiriguá	67	26.9	65	35.4	1.11	−0.14
Quiriguá, Praia Grande	Praia Grande	65	75.4	66	56.1	5.48	0.15
Quiriguá, Praia Grande	Quiriguá	90	24.4	92	62.0	25.87	−0.44

Table 1 shows that no significant deviations from randomness of mating are observed between any of the Brazilian strains (Praia Grande, Bertioga, Rio, Belem). The highest χ^2 value for such matings is 5.49, which, for 1 degree of freedom, indicates the probability of about 0.02 of the deviation being due to chance. Summing up the results of the Brazilian crosses, we have 62% of homogamic and nearly 60% of heterogamic inseminations among 1090 females dissected, the difference being statistically non-significant. A different situation obtains when the Guatemalan strain (Quiriguá) is crossed to the Brazilian ones. The isolation index is positive whenever the males used are Brazilian, and negative when Quiriguá males are used. This suggests that when a mixture of Brazilian and Guatemalan

females is offered either to Brazilian or to Guatemalan males, Brazilian females are inseminated in preference to the Guatemalan ones. To be sure, the observed deviations from random insemination are mostly not significant statistically (the χ^2 values corresponding to the 0.05, 0.02, and 0.01 probability levels are 3.84, 5.41 and 6.64, respectively). An unquestionably significant positive isolation index is observed only when Bertioga males are offered a choice of Bertioga and Quiriguá females, and a negative isolation index only in the cross of Quiriguá and Praia Grande females to Quiriguá males. Combining the data for the crosses in which Brazilian males were offered a choice of Brazilian and Guatemalan females, we find the frequencies of the homo- and heterogamic matings to be 62.2% and 34.9%, respectively, with a χ^2 of 39.80, which is very significant. A similar summation of the data for the crosses of Guatemalan males to mixtures of Guatemalan and Brazilian females gives 32.9% for homogamic and 48.2% for heterogamic matings; χ^2 is 13.68 corresponding to about the 0.001 probability level.

TABLE 2

NUMBER OF FEMALES DISSECTED (n) AND PER CENT CARRYING SPERM (%) IN CROSSES MADE IN QUART-SIZED BOTTLES

FEMALES	MALES	HOMOGAMIC n	HOMOGAMIC %	HETEROGAMIC n	HETEROGAMIC %	χ^2	ISOLATING INDEX
Bertioga, Belem	Bertioga	107	73.8	107	69.2	0.57	0.03
Quiriguá, Rio	Rio	192	54.2	198	39.4	4.55	0.16
Quiriguá, Praia Grande	Quiriguá	101	49.5	105	76.2	15.29	−0.21

The degree of preference for homo- or heterogamic mating is probably subject to environmental modification. An unpublished experiment of Professor W. P. Spencer suggested that the amount of space available to the flies may influence the degree of mating preference (personal communication). To test the results of the main body of experiments, in which 30 flies were confined in $3^3/_4$- × 1-inch vials, some crosses were repeated using 40 females of each of two strains and 40 males of one of them in quart milk bottles. Some culture medium was poured on the bottom of the bottles, and crumpled paper toweling was placed so that the entire space in the bottle was easily accessible to the flies. The results are summarized in table 2. They fully confirm the conclusions reached previously. No significant deviation from randomness of mating is observed when Bertioga males are given a choice of Bertioga and Belem females (Brazilian × Brazilian mating). Brazilian (Rio) males inseminate more Brazilian than Guatemalan females. Guatemalan males likewise inseminate more Brazilian than Guatemalan females.

The writers take the pleasure of acknowledging their obligations to Miss Irene Markreich for her assistance in conducting the experiments.

Summary.—With the exception of the Teffé strain which will be discussed in another publication, no trace of sexual isolation is observed between strains of *D. willistoni* from different parts of Brazil and from Guatemala. When Brazilian males are offered a choice of females of their own and of other Brazilian strains, inseminations occur at random. If a mixture of Brazilian and Guatemalan females are confined either with Brazilian or with Guatemalan males, a greater proportion of Brazilian than of Guatemalan females are inseminated. The mating of Brazilian and Guatemalan flies is, therefore, selective rather than random; however, the particular type of selectivity here observed does not constitute a barrier to gene exchange.

[1] Dobzhansky, Th., *Amer. Natur.*, **71**, 404–420 (1937).

[2] Dobzhansky, Th., *Genetics and the Origin of Species*, New York, 1937, 1941.

[3] Mayr., E., *Systematics and the Origin of Species*, New York, 1942.

[4] The expression "physiological isolating mechanisms" used in some papers of Dobzhansky was intended to distinguish between isolating mechanisms based on inherent properties (physiology) of the organisms concerned and geographical isolation. Since this expression has led to some misapprehension, the adjective "reproductive," instead of "physiological," is used to convey the same idea.

[5] Phillips, J. C., *J. Exp. Zool.*, **18**, 69–146 (1915).

[6] Gordon, M., *Trans. N. Y. Acad. Sci.*, **5** (2), 63–77 (1943).

[7] Dobzhansky, Th., and Koller, P., *Biol. Zentr.*, **58**, 589–607 (1938).

[8] Stalker, H. D., *Genetics*, **27**, 238–257 (1942).

[9] Patterson, J. T., Stone, W. S., and Griffen, A. B., *Univ. Texas Public.*, **4228**, 162–200 (1942); Stone, W. S., *Ibid.*, 16–22; Griffen, A. B., *Ibid.*, 68–73; Mainland, G. B., *Ibid.*, 74–112.

[10] Copulation in *D. willistoni* begins as early as 8 hours after the hatching from pupae; to collect virgins bottles were emptied at 4-hour intervals, but it was found expedient to use also females hatched overnight since the proportion fertilized is small.

THE COMPRESSIBILITY OF MEDIA UNDER EXTREME PRESSURES

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It is well known that Hooke's Law, which postulates a linear relation between stress and strain, has a very limited range of applicability even when the applied stress is a uniform pressure. We have in previous papers[1,2] furnished a formula which is valid over a much greater range than Hooke's Law; this formula agreed well with experimental results up to pressures as high as 50,000 atmospheres (the highest for which measurements were

then available). Since then Bridgman[3] has published measurements up to 100,000 atmospheres and this, combined with theoretical considerations of a fundamental character, has caused us to reconsider the whole question. Hooke's Law and our formula may both be derived from the principle of energy conservation; the difference in the results is due to the fact that for infinitesimal deformations (to which the validity of Hooke's Law is limited) it is correct to say that stress is the gradient, with respect to strain, of the energy per unit volume. For non-infinitesimal deformations this simple relation must be replaced by a more complicated one. The feature common to both theories is the *assumption* that the energy density is a function of the strain; it being agreed that the strain is measured from the position of zero stress. This assumption, which has apparently never been seriously questioned, has the quality of an "action at a distance" theory; we assume that the energy of deformation is furnished by a knowledge of the relationship of the actual position of the medium to a *remote* position in which the medium was unstressed. We propose here to discard this action at a distance theory and to replace it by a *differential* theory in which we are concerned merely with the variation of the energy as we pass from *any* position of the medium to an infinitesimally near position. Thus we must be prepared to confront the situation where the *initial* position of the medium is one in which the medium is under stress.

This being understood, a fundamental question arises: are the elastic *constants* of the medium really constant? The elastic constants are simply coefficients which occur in the statement of Hooke's Law and we mean by the word *constant* that they do not depend on the *increment* of the stress. But do they depend on the initial value of the stress? All experimental evidence points to the fact that they do, and many determinations have been made of the variation of such things as compressibility with pressure. It is fair to say that the reason that Hooke's Law is so limited in its range of applicability is that it neglects the dependence of the elastic constants on the initial stress. This dependence of the elastic constants on the initial stress cannot be overemphasized. For example, when we say that an elastic medium is isotropic, we mean that certain relations hold amongst the elastic constants (so that *two* are sufficient to furnish the statement of Hooke's Law); it is clear, then, that a medium which is elastically isotropic under zero stress may fail to be elastically isotropic when stress is applied. We should certainly expect this lack of isotropy to appear when the applied stress is (as in the case of the Young's modulus experiment) not merely a uniform pressure or traction. We feel certain that it is the reliance of the classical theory of elasticity upon the hope that a stretched wire is elastically isotropic, if the unstretched wire is, that is responsible for its complete failure to predict the yield point phenomenon.

The case of uniform pressure is particularly simple since the stress tensor

is scalar. The principle of conservation of mass, combined with Hooke's law for *infinitesimal* variations of stress, suffices provided we are willing to face the situation that the compressibility depends on the pressure. The principle of mass conservation yields $\frac{1}{v}\delta v = 3\frac{\partial}{\partial x}\delta x$ and Hooke's Law yields $p = -(3\lambda + 2\mu)\frac{\partial}{\partial x}\delta x$ and so

$$\frac{1}{v}\frac{dv}{dp} = -\frac{1}{\lambda + {}^2/_3\mu}.$$

Let us assume, as a first approximation, that $\lambda + {}^2/_3\mu$ is a linear function of p:

$$-v\frac{dp}{dv} = c(1 + kp)$$

so that

$$c = -\left(v\frac{dp}{dv}\right)_{p=0}; \qquad ck = -\frac{d}{dp}\left(v\frac{dp}{dv}\right)_{p=0}.$$

We find $\frac{v_0}{v} = (1 + kp)^{1/ck}$ or, on writing $\Delta v = (v_0 - v)$,

$$\frac{\Delta v}{v_0} = 1 - (1 + kp)^{-1/ck}.$$

This simple formula accounts reasonably well for the experimental results even for such a range of pressure as from 0 to 100,000 atmospheres. The data for Li given by Bridgman (loc. cit.) are approximated by setting $k = 0.153 \times 10^{-4}$, $ck = 2$ so that the relation connecting p and v has the simple form

$$0.153 \times 10^{-4}p = \left(\frac{v_0}{v}\right)^2 - 1; \qquad \frac{\Delta v}{v_0} = 1 - (1 + 0.153\cdot 10^{-4}p)^{-1/2}.$$

Bridgman tabulates $\frac{\Delta v}{v_0}$ at intervals of 10,000 atmospheres. The following table furnishes the comparison between the values obtained empirically and those calculated from the formula just given

$p =$	10^4;	2.10^4;	3.10^4;	4.10^4;	5.10^4;	6.10^4;	7.10^4;	8.10^4;	9.10^4;	10^5
$\frac{\Delta v}{v_0}$ (obs.)	0.074;	0.125;	0.164;	0.201;	0.237;	0.272;	0.305;	0.336;	0.366;	0.394
$\frac{\Delta v}{v_0}$ (calc.)	0.069;	0.125;	0.172;	0.212;	0.247;	0.278;	0.305;	0.330;	0.353;	0.371

The formula predicts that when $p = 2.10^5 \frac{\Delta v}{v_0}$ will be approximately 0.514; when $p = 10^6 \frac{\Delta v}{v_0}$ will be approximately 0.752. To compress Li to a density ten times its original density, a pressure of around 6.5×10^6 atmospheres is necessary.

In conclusion we add the remark that when the pressure is small enough so that its influence upon the elastic constants is negligible the appropriate formula is

$$\frac{v_0}{v} = e^{p/c}; \qquad \frac{\Delta v}{v_0} = 1 - e^{-p/c}.$$

ADDED IN PROOF. It is worthy of attention that the formula given here is, when the dimensionless constant ck is assigned the value 2, precisely that which leads to Laplace's well known law of density distribution throughout the interior of the earth. This law fits the observed facts remarkably well but it has been regarded by many authorities as merely empirical largely because its connection with the law of compressibility has not been clearly presented.

[1] "Finite Deformations of an Elastic Solid," *Amer. Jour. Math.*, **59**, 235–260 (1937).

[2] "The Compressibility of Solids," *von Kármán Anniversary Volume*, 1941, pp. 121–136.

[3] "Compressions and Polymorphic Transitions of Seventeen Elements to 10^5 Kg./-Cm.^{2}," *Phys. Rev.*, **60**, 351–354 (1941).

NOTE ON THE DISCOVERY OF RED STARS

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During an investigation of colors, magnitudes, and spectral types in the Hercules-Vulpecula region, it was noted that a star at the approximate position 19^h10^m6, $+21° 37'$ (1855), $\lambda = 23°$, $\beta = +3°$ had a very large color index. A preliminary examination of blue and red plates indicated that the blue-red color index was between 6.5 and 7.0 magnitudes.

The blue-red and blue-yellow (international) color indices of this star were determined from polar comparison plates, the blue magnitude from Cramer Hi-Speed plates, the red magnitude from Eastman 103a-E plates with a ciné-red filter (effective wave-length near 6300 A), the yellow magnitude from 103a-G plates with a yellow filter. The 8-inch Ross-Lundin (IR) refractor was used for the red and the yellow, the 16-inch Metcalf (MC) refractor for the blue magnitudes. All plates were taken between May 26 and June 5, 1944. The results were as follows:

Mag_{pg}	$14^{m}.75$
Mag_{pr}	$8^{m}.10$
Mag_{pv}	$9^{m}.75$
C.I. (blue-red)	$6^{m}.65 \pm 0.10$
C.I. (blue-yellow)	$5^{m}.00 \pm 0.10$

This star has been observed before by Lee and his associates; it is No. 129 in the list of Dearborn Annals, Vol. IV, Part 16, 1940, where its spectral type is given as *N*.

The star is not unlike the very red star discovered in 1935 by F. K. Edmondson and A. M. Rogers.[1] Both Edmondson's star, BN Monocerotis, and the present object are variable.[2] An examination of plates in the Harvard Collection gives the following provisional results:

DATE	MAG_{pg}
Aug. 31, 1897	13.55
Sept. 11, 1917	15.10
July 5, 1926	13.80
Oct. 28, 1927	14.30
Apr. 18, 1928	14.35
Oct. 9, 1928	14.10
June 3, 1929	13.70
July 15, 1929	13.60
July 18, 1930	14.00
May 11, 1937	13.90
July 13, 1937	13.70
May 28, 1938	13.60
May 30, 1938	13.60
Aug. 30, 1940	14.60
May 26, 1944	14.80
May 28, 1944	14.85
May 29, 1944	14.75

The finding of this very red star led the author to attempt a search for other very red stars in the same region of the sky. The method employed in this search resembles that used previously by several astronomers, among others, G. A. Tikhov,[3] V. M. Slipher[4] and G. Z. Dimitroff.[5]

The instrument used in the survey was again the 8-inch Ross-Lundin (IR) refractor. The region was photographed on an Eastman 103a-E plate, exposure time five minutes at normal blue focus and without filter. Since the IR camera has a very steep focal curve, ($^{1}/_{6}$ inch distance between blue and red focus) and the 103a-E plates are sensitive to the blue as well as to the red region of the spectrum, the red stars appear as a black dot (blue) surrounded by a strong red halo, when focused for the blue. The halo is almost totally absent for the very blue stars, but quite conspicuous for the normal late-type stars. The above-mentioned star is so red that the blue

central dot is absent on a five-minute exposure; there appears on the plate only a halo of fairly uniform brightness with a central hole.

The region was photographed a second time with the IR camera, blue focus, 103a-E plates, no filter, and with an exposure time of thirty minutes. This plate revealed the presence of three additional very red stars, marked by a red halo without the central blue image. The approximate positions, magnitudes and color indices of these stars are:

	STAR 2	STAR 3	STAR 4
R.A. (1855)	$19^h\ 9^m.2$	$19^h\ 8^m$	$19^h\ 9^m$
Dec. (1855)	+24° 58′	+18° 20′	+17° 10′
λ	26°	18°	17°
β	+5°	+3°.5	+3°
Mag_{pg}	16.40	15.60	15.70
Mag_{pr}	9.90	10.45	10.35
Mag_{pv}	11.50		
C.I. (blue-red)	6.50 ± 0.10	5.15 ± 0.10	5.35 ± 0.10
C.I. (blue-yellow)	4.90 ± 0.10		

The spectral type of the second star was determined from two plates taken by Miss Florence Anderson with the 12-inch Metcalf (MA) refractor, exposure time two hours. The spectrum is faint, and resembles that of an *N* star.

Following the search of the first field, discovery plates were made for two additional centers, one eight degrees north, the other eight degrees south of the first center, with thirty minutes' exposure time. An examination of these two plates reveals the presence of four additional conspicuously red stars, with a red halo, but no central blue image. It seems likely that the blue-red color index of each of these stars is well in excess of four magnitudes. The approximate 1855 positions from BD-charts are:

STAR	R.A. (1855)	DEC. (1855)	λ	β
5	$18^h\ 58^m.3$	+13° 30′	13°.5	+2°.5
6	19 11	+ 8 35	9	−1.5
7	19 10	+27 36	28	+6.5
8	19 16.3	+31 2	31	+7

The total area covered by the present survey is approximately 250 square degrees; it is complete to the eleventh photo-red magnitude. The survey yields eight stars with color indices (blue-red) of more than four magnitudes. It should be noted that all stars are within eight degrees of the galactic circle. A study of color indices of stars of known spectral type shows the average absorption for the region to be of the order of one magnitude per kiloparsec. None of the stars is found in a region of heavy obscuration included in the survey.

[1] *Pub. A.S.P.*, **47**, 191 (1935).
[2] *Ap. Jour.*, **100**, 1 (1944).
[3] *A.N.*, **218**, 145 (1922) and *Poulkovo Publ.*, Ser. 2, **50** (1937).
[4] *Pub. A.A.S.*, **7**, 15 (1931).
[5] *The Telescope*, **6**, 62 (1939).

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*ANTIBIOTIC ACTIVITY OF LICHENS**

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The production by an organism of organic substances inimical to the life processes of other organisms and inhibiting their growth is known as antibiosis. The phenomenon of antibiosis has attracted wide-spread attention in recent years chiefly because of the remarkable chemotherapeutic effects of penicillin, a product of the mold *Penicillium*. The anabolic products of many kinds of bacteria,[1] molds,[2] and algae,[3] and substances elaborated by certain species of flowering plants[4] can be readily demonstrated to have antibiotic properties when small amounts of the active materials are tested on microörganisms with suitable assay procedures. In view of the reported antibacterial activity of the green alga *Chlorella* and the many antagonistic substances now known to be produced by numerous kinds of fungi, the lichens seemed to offer favorable material for antibiotic investigations, inasmuch as the bodies of these plants are comprised of mixtures of algae and fungi. Accordingly we have tested a considerable number of lichens for antagonistic action against some common species of bacteria, and offer herewith a preliminary report concerning some of our observations.

Methods.—The lichens were brought in fresh condition to the laboratory, placed in shallow pans, and moistened with water for the purpose of allowing them to become physiologically more active. After a few hours of exposure to sunlight, samples of the moist lichens were separated from the substrata with a razor blade and forceps. Extracts were made by grinding, with a glass mortar and pestle, 100 mg. of lichen in 1 ml. of phosphate buffer solution adjusted to pH 7.4. The final aqueous extract was approximately pH 7.0. Extractions were carried out at room temperature and the samples were stored at 1°C. for a short time until the materials could be assayed. No attempt was made to clarify the extracts in these preliminary tests. The aqueous suspensions were assayed against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* or other bacteria by means

of the cylinder plate procedure.[5] As is well known to workers in this field, the method involves the use of agar plate cultures of bacteria upon which are placed small glass cups to which are added the assay liquids. After a period of incubation, zones of bacterial growth-inhibition subjacent to the cups are found to vary in diameter depending upon the potency of the test solutions which have diffused out of the cups into the agar. In these preliminary tests with aqueous suspensions the diameter of the zones of inhibition ranged up to 22 mm.

TABLE 1

ANTIBIOTIC ACTIVITY OF EXTRACTS OF LICHENS IN PHOSPHATE BUFFER TESTED AGAINST THREE BACTERIA. + = INHIBITED; − = NOT INHIBITED

SPECIES OF LICHEN	*S. aureus*	*E. coli*	*B. subtilis*
C. caespiticia	+	−	+
C. capitata	+	−	+
C. coniocraea	+	−	+
C. cristatella	+	−	+
C. glauca	+	−	+
C. Grayi	+	−	+
C. nemoxyna	+	−	+
C. squamosa	+	−	+
C. strepsilis	+	−	+
Parmelia physodes	+	−	+
Physcia sp.	+	−	+
Ramalina sp.	+	−	+
Sterocaulon paschale	+	−	+
C. incrassata	−	−	+
C. atlantica	−	−	+
C. subtenuis	−	−	+
C. caroliniana	−	−	+
C. uncialis	−	−	+
Lepra sp.?	−	−	+
Parmelia conspersa	−	−	+
Peltigera sp.	−	−	+
C. furcata	+	−	−
C. papillaria	+	−	−
Umbilicaria papulosa	+	−	−

Further experiments were performed with selected species of lichens for the purpose of determining whether the active compounds could be extracted with solvents, such as ether, chloroform and ethyl alcohol. The moist lichen materials were ground in a glass mortar, using 100 mg. of lichen to approximately 1 ml. of solvent. The suspensions were then centrifuged, and the clear solutions were pipetted into small flasks which could be attached to a vacuum distillation apparatus for removing the solvents at low temperature. The residual matter was taken up in aqueous phosphate buffer at pH 7.4, and after mixing with a glass rod the material was

tested in the cup plates. Incubation of the test plates was allowed to proceed for 12 hours at 37°C.

Results.—Of the 42 species of lichens tested thus far, 27 have been found active against either *S. aureus* or *B. subtilis*. Some of these are listed in table 1. In addition to those given in the table, *Cladonia cryptochlorophaea*, *C. pyxidata* and *Dermatocarpon miniatum* were found to inhibit *S. aureus*, but were not tested with *B. subtilis*. None of the 42 lichens which were tested shows any activity against *E. coli*. Slight inhibition against *Alcaligenes fecalis* was noted for the lichen species *C. glauca* and *Parmelia conspersa*. A strain of *Proteus vulgaris* was inhibited by *Parmelia physodes* and by *Umbilicaria papulosa*. *Aerobacter aerogenes* and *Serratia marcescens* were not inhibited by the lichens which were antibiotic for *Alcaligenes* and *Proteus*. The lichens *C. atlantica*, *C. caroliniana*, *C. cristatella*, *C. Grayi*, and *Parmelia physodes*, which are active against *B. subtilis*, were tested also with *B. cereus* and *B. vulgatus*, and were found inhibitory for these species of bacteria. Species which were inactive when tested with *E. coli* and *S. aureus* are the following: *Baeomyces roseus*, *C. apodocarpa*, *C. bacillaris*, *C. clavulifera*, *C. conista*, *C. pleurota*, *C. Robbinsii*, *C. subcariosa*, *C. submitis*, *C. verticillata*, *Leptogium* sp., *P. rudecta* and *Parmeliopsis aleurites*. *Baeomyces roseus*, *C. apodocarpa* and *C. subcariosa* were inactive also with *B. subtilis*. It is possible that activity would be found if these lichens were tested against other bacteria.

The activities of different lichens which were extracted with chloroform, ethyl alcohol or ether are recorded in table 2. The equivalent weights of moist lichen material (200, 300 or 500 mg.) extracted with the solvents and finally taken up in 1.0 or 1.2 ml. of phosphate buffer solution are listed under each species in the table. Although the data are not strictly quantitative for chloroform and alcohol because of some losses incurred during transfer of the solutions, the results indicate clearly that active antibacterial substances can be extracted from lichens with convenient solvents and passed on into an aqueous test medium. That more than one antibiotic compound may exist in lichens is suggested by the fact that both *S. aureus* and *B. subtilis* are inhibited by extracts from *C. Grayi*, *Parmelia physodes* and other species, while substances obtained from some species of *Cladonia* inhibit *B. subtilis* but not *S. aureus*. Furthermore, extracts from *C. furcata*, *C. papillaria* and *Umbilicaria papulosa* inhibit *S. aureus* but are inactive against *B. subtilis*. The inhibition of some Gram-negative bacteria by selected species of lichens lends further support to the theory of multiple substances. Until more information becomes available about the nature of these antibacterial compounds, no names will be suggested for them as additions to the already confused terminology of antibiotics.

Characteristic zones of bacterial inhibition obtained with ether extracts, transferred to phosphate buffer for making the tests, are shown in figure 1. It should be mentioned here that with some lichens inhibition was obtained with both ether and aqueous extracts; in other instances antibiosis was found with only the ether or the phosphate buffer solution. As an example, an ether extract of *C. glauca* in figure 1 shows no antag-

TABLE 2

ANTIBIOTIC ACTIVITY OF LICHEN EXTRACTS TESTED AGAINST *B. subtilis*. WEIGHT OF MOIST LICHEN IS GIVEN AS MG. PER ML. OF BUFFER SOLUTION. DIAMETERS OF BACTERIAL INHIBITION ZONES ARE EXPRESSED IN MM.

SPECIES OF LICHEN	PRIMARY SOLVENT	INHIBITION ZONES
C. caroliniana	Chloroform	23
(500 mg./1.2 ml.)	Alcohol	19
	Ether	25
C. cristatella	Chloroform	26
(200 mg./1.2 ml.)	Alcohol	27
	Ether	28
C. incrassata	Chloroform	25
(200 mg./1.2 ml.)	Alcohol	25
	Ether	29
C. Grayi	Chloroform	23
(300 mg./1.2 ml.)	Alcohol	24
	Ether	25
Parmelia physodes	Chloroform	15
(200 mg./1.2 ml.)	Alcohol	15
	Ether	18
C. strepsilis	Ether	20
(200 mg./1.0 ml.)		
C. nemoxyna	Ether	20
(200 mg./1.0 ml.)		
Parmelia conspersa	Ether	23
(200 mg./1.0 ml.)		
Peltigera sp.	Ether	16
(200 mg./1.0 ml.)		
Physcia sp.	Ether	23
(200 mg./1.0 ml.)		
Ramalina sp.	Ether	21
(200 mg./1.0 ml.)		
C. uncialis	Ether	19
(200 mg./1.0 ml.)		

onism for *B. subtilis*, but an aqueous extract made directly from the same lichen gave slight inhibitory action on the same species of bacteria. Hence we have listed *C. glauca* in table 1 as being active against *B. subtilis*.

Without additional information it is impossible to say whether the antibiotic activity of lichens should be attributed to the algal or fungal member of the complex. Almost nothing is known about the anabolism of the

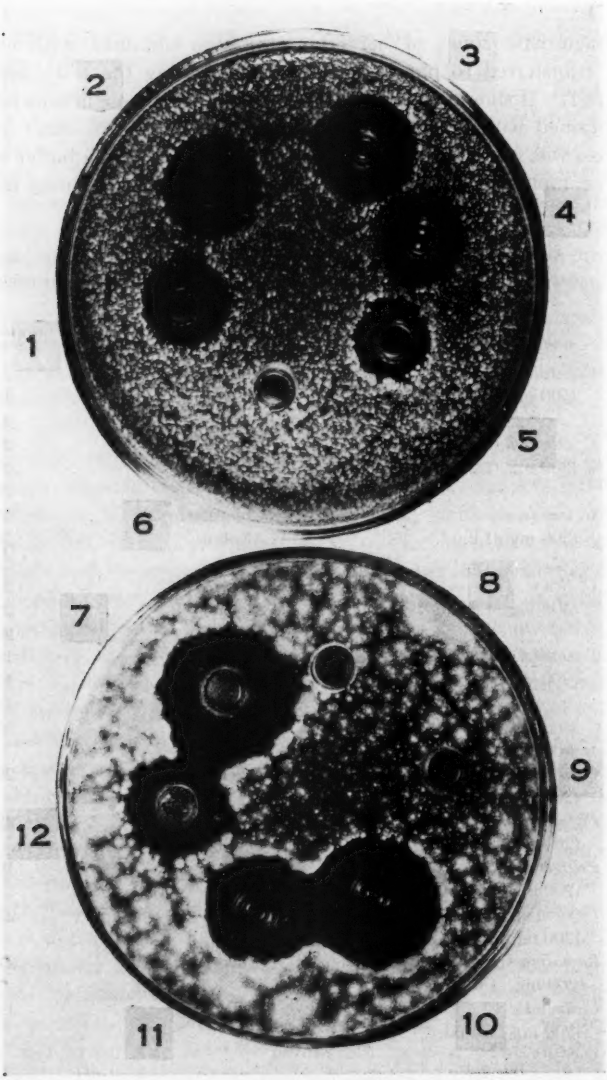

FIGURE 1

Antibacterial action of extracts of lichens. Above, lichen substances extracted with ether were tested in aqueous phosphate buffer against *B. cereus;* below, extracts tested against *B. subtilis*. Sources of the extracts: 1, *Parmelia physodes;* 2, *C. cristatella;* 3, *C. caroliniana;* 4, *C. atlantica;* 5, *C. Grayi;* 6, *C. coniocraea;* 7, *Parmelia conspersa;* 8, *C. apodocarpa;* 9, *C. glauca;* 10, *C. caroliniana;* 11, *Physcia* sp.; 12, *Ramalina* sp.

components or the roles of the various substances formed in the lichen body. Chemical antagonism would appear to constitute a possible defense mechanism operating in the struggle for existence among these primitive plants.

One question of considerable importance emerges from the data reported in this paper when considered in the light of earlier work done on the structural chemistry of the lichen acids by Zopf,[6] Asahina,[7] and others. Do the characteristic lichen acids possess antibacterial activity or are the antibiotic properties of lichens related to traces of other unidentified substances synthesized by these plants? Some of the known lichen compounds possess certain structural features in common with antibacterial substances which have been isolated from molds, but whether these are responsible for the antibiotic phenomena which we have observed can be tested only by further experiments which are in progress.

Summary.—Antibacterial activity of extracts from 42 species of lichens was determined with the cup plate procedure. Twenty-seven species were found active against *S. aureus* or *B. subtilis*, 4 species inhibited *P. vulgaris* or *Alcaligenes fecalis*, but none showed antagonism against *E. coli*.

* This work was supported in part by a research grant from Parke, Davis & Company.

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ACTIVITY AND NUTRITIONAL DEPRIVATION

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Animals in general exhibit increased activity when in want of food. This is in fact the indispensable sign of hunger. It has been observed in widely different organisms from mammals to protozoa;[1] in man and other animals during sleep;[2] in dogs,[3] guinea pigs,[4] and pigeons[5] after decerebration; and in rats from which virtually the entire stomach had been removed.[6] Increased activity therefore holds a primary position in the hunger reaction, persisting in higher animals which lack the equipment for either hunger sensations or the hunger contractions of the stomach.

That it has this status is not extraordinary, since in all animals feeding depends upon either moving about in the environment or, in certain aquatic forms, stirring the environment, in either case bringing the animal into contact with its food.

Sexual demands may evoke behavorial reactions similar to those as-

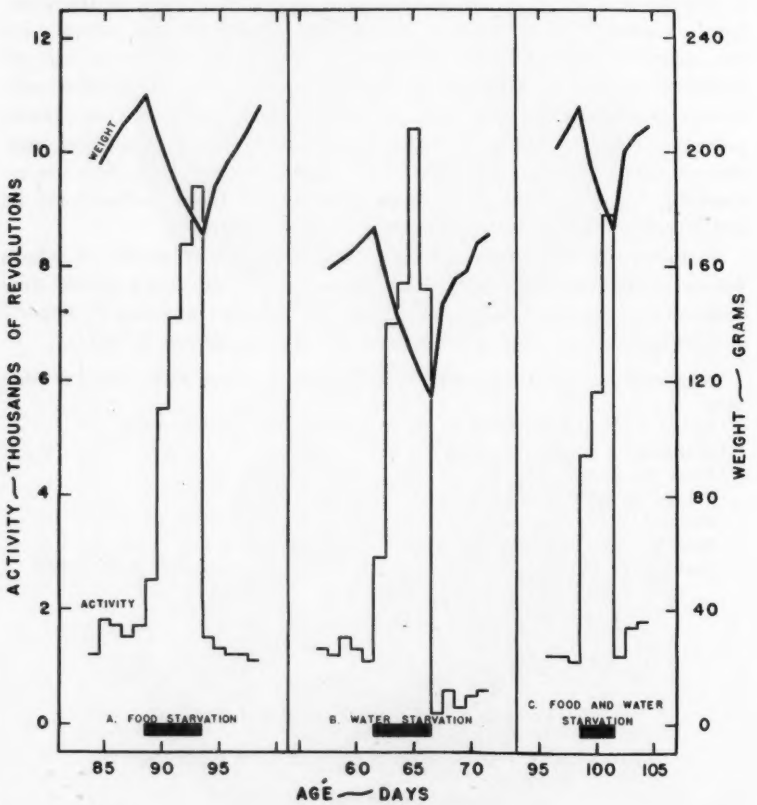

FIGURE 1

Running of the rat in food and water deprivation. Effects on the average weight and daily running of deprivation and readministration of total dry food (*A*, 12 animals); water (*B*, 3 animals); and food and water (*C*, 12 animals). In each figure the black area under the baseline represents the period of deprivation.

sociated with nutritional need. Female rats, for example, display a high peak of running when in oestrus and receptive to the male. This ceases on copulation or artificial stimulation of the cervix uteri.[7]

Several years ago we began experiments on the spontaneous running of

white rats in nutritional deprivation. Male animals were kept in activity cages in which they had free access to a running wheel (1440 revolutions to the mile).[8] In this arrangement they normally ran an average of 0.4–1.2 miles daily.

A rat deprived of its bulk food while given water *ad libitum* greatly increases its running. On readmission of food the high running ceases and the rat begins to feed vigorously (Fig. 1 *A*). Rats allowed free access to their dry food but deprived of water react similarly (Fig. 1 *B*), as do also animals deprived of both food and water (Fig. 1 *C*).

To the degree that these responses depend upon genuine tissue needs and not simply upon mechanical factors such as the filling and emptying of the stomach, one might expect them to be elicited by single indispensable components of the diet. Water itself is such a component. Thiamin also appears to have this status.

Responses to the deprivation and readministration of thiamin are shown in figure 2.[9] They are of precisely the same form as those associated with variations in the supply of bulk food or water. Some time after our initial experiments were performed we learned that high running in thiamin deprivation had already been reported by Guerrant and Dutcher;[10] recently Bloomfield and Tainter[11] have observed similar responses in rats deprived of the entire vitamin B complex.

In rats continued on a thiamin-deficient diet maximal running persists for about 10 days. Then it falls within a further 2–8 days to far below the normal level (Fig. 3). Simultaneously the classic neuromuscular symptoms of thiamin deficiency appear: spasticity of the limbs, hunching of the back and finally acute polyneuritis.[12] Usually animals do not run below the normal level for more than a day before developing polyneuritis. The rat of figure 3, for example, in repeated trials regularly ran about 3000 revolutions on the very day before it became polyneuritic and appeared to be moribund.

From this terminal condition the animal may be rapidly restored with a single injection of thiamin. Its immediate response—beyond the cure of polyneuritis—is to eat enormously. Meanwhile it runs no more and may run even less than when polyneuritic. The weight rises rapidly for a time, then levels off. At this point the animal again begins to run, and reaches a peak of activity as eating and weight decline. Each injection of thiamin initiates such connected cycles of eating, weight-change and running, rising to maxima in that order, and returning finally to the minimal levels at which the rat is again polyneuritic (Fig. 3).

A striking interplay between thiamin and bulk food deprivation may be demonstrated as follows (Fig. 3). Food consumption falls during thiamin deficiency from normal daily values of 10–14 grams to about 2–3 grams prior to polyneuritis. If a polyneuritic animal is injected with large

daily doses of thiamin and is meanwhile restricted to the low food intake it had established on the thiamin-low diet, it begins to run enormously. Thiamin, in addition to curing its polyneuritis, has restored its hunger for bulk food to a degree which the restricted allotment no longer satisfies.

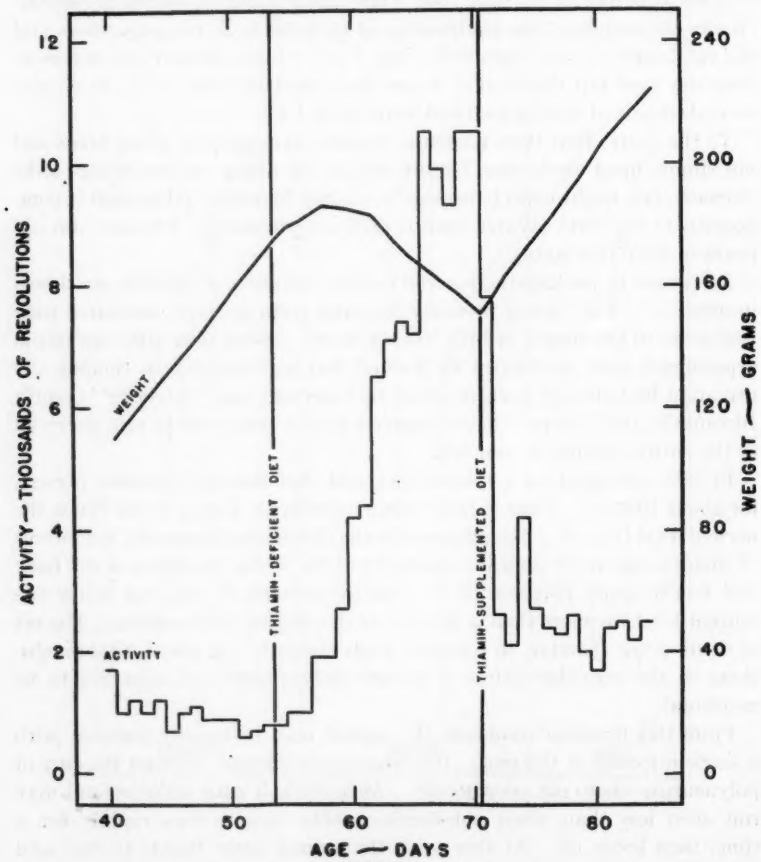

FIGURE 2

Effects of deprivation and readministration of thiamin. Averaged data from 6 animals. From 54 to 71 days of age, the thiamin supplement was omitted from an otherwise complete diet.

Specific thiamin-hunger has been replaced by the hunger for bulk food. In consequence the animal runs, and in an amount that its limited caloric intake cannot support. Having already been reduced during thiamin deficiency to an extreme state of inanition, it usually dies after a few days of great activity.

The effects of deprivation and readministration of riboflavin in rats on an otherwise complete diet are shown in figure 4. They are essentially similar to the responses already described, though not usually as prompt or marked as those associated with the thiamin nutrition.

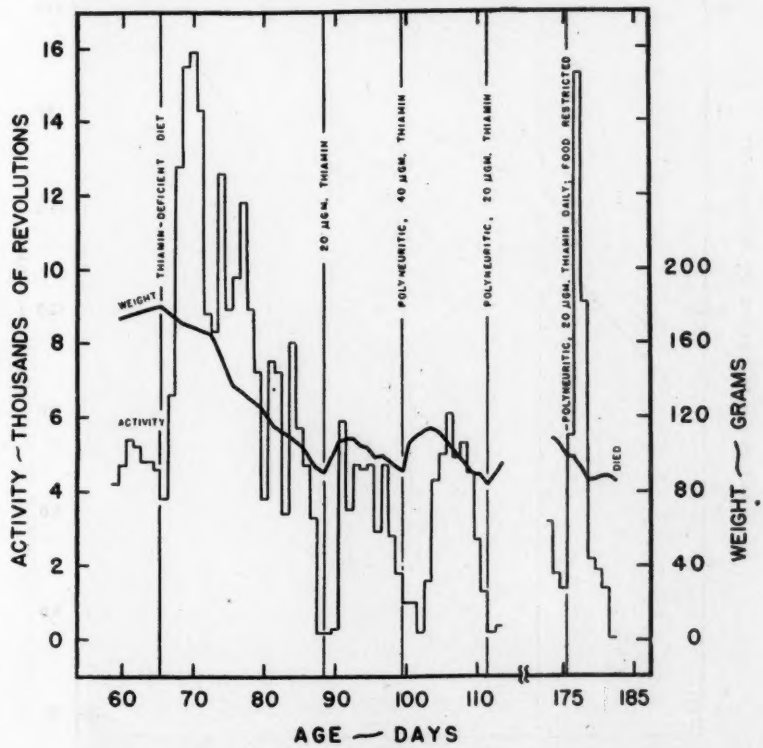

FIGURE 3

Weight and daily running of a single animal deprived of thiamin when 65 days old. On its 88th day and at intervals thereafter it was injected intraperitoneally with the amounts of thiamin indicated, being meanwhile continued on the thiamin-low ration. On the 176th day and thereafter it was injected daily with 20 μg. thiamin and was restricted meanwhile to the amounts of ration consumed while thiamin-deficient.

We have examined also the effect of depriving rats of magnesium ion (Fig. 5). In this case no high running whatever is observed. Instead the running declines after 1–2 weeks of deprivation to very low values. Magnesium is one indispensable component of the diet, therefore, the lack of which does not stimulate increased activity. This appears to be true also of deprivation in total inorganic ions,[13] or in vitamin A.[14]

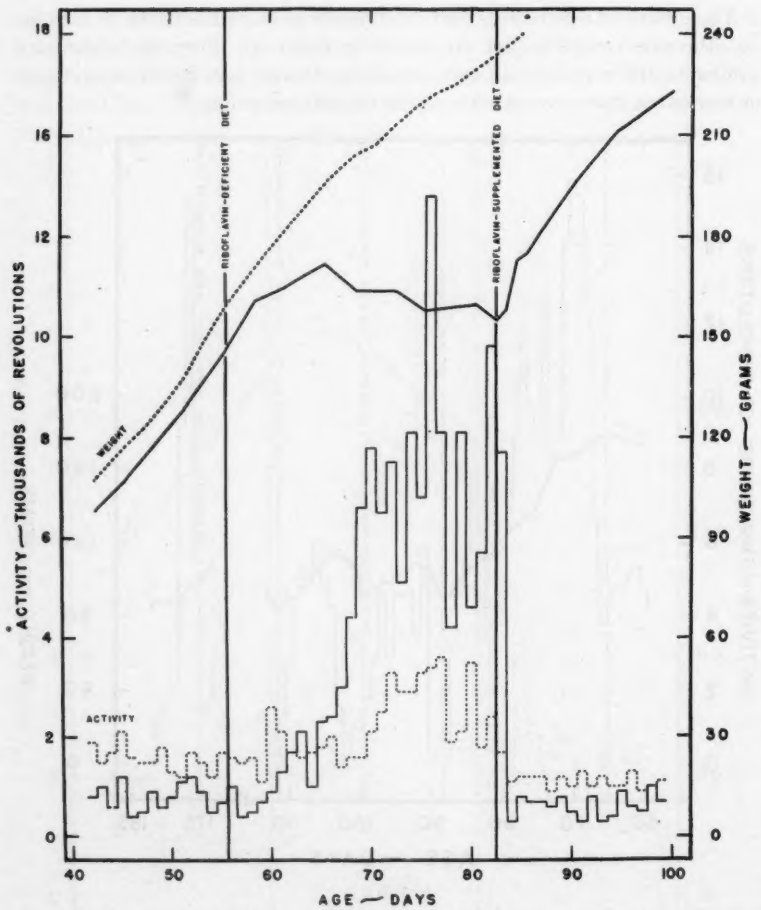

FIGURE 4

Deprivation and readministration of riboflavin. Data from a single experimental rat (solid lines) compared with averaged measurements on 5 control animals (broken lines). From age 56 to 83 days the riboflavin supplement was omitted from the diet of the experimental animal.

It may be concluded that increased running is a basic response to nutritional need, and that it may be stimulated by the lack of specific indispensable components of the diet. It is noteworthy that, except for restriction of water or of the bulk of solid food, the only types of deprivation which

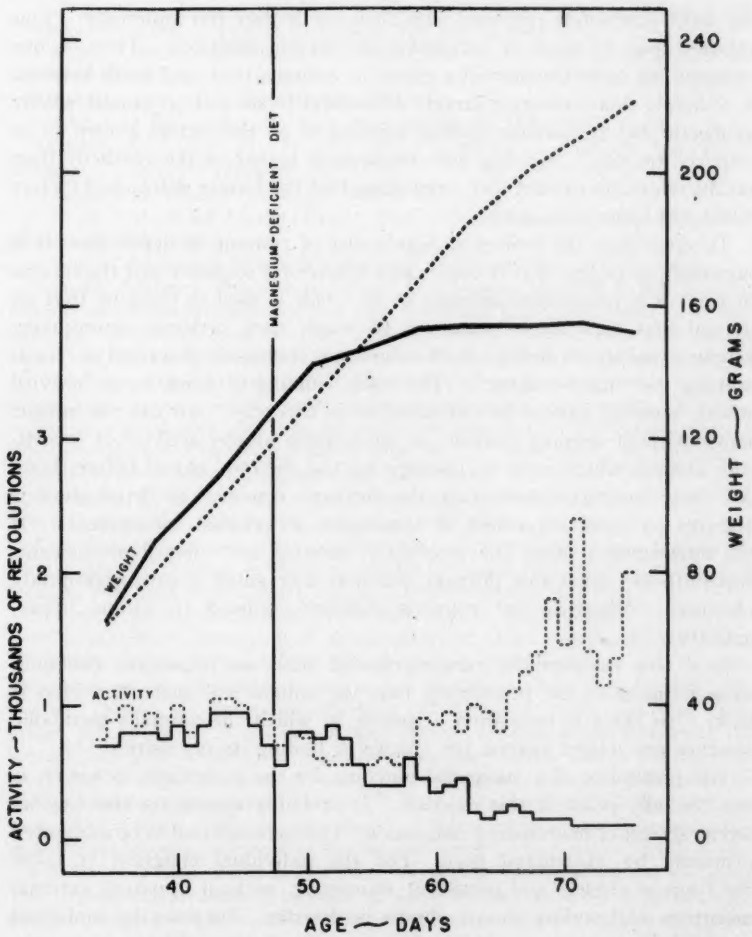

FIGURE 5

Deprivation of magnesium ion. Averaged data from 5 experimental (solid lines) and 5 control animals (broken lines). After the 47th day the magnesium supplement was omitted from the otherwise complete diet of the experimental animals.

have as yet been shown to result in increased running have included deprivation in members of the vitamin B complex.

High running is not, therefore, a reliable sign of well being and optimal performance. It may be a sign of want. When healthy, intact animals

are most completely provided with their needs they run minimally. This relation may be used as a criterion of dietary adequacy. Thus on one occasion we have transferred a group of animals back and forth between a complete diet consisting largely of natural foods and an almost wholly synthetic diet containing optimal supplies of all the factors known to be required by rats. Running was consistently higher on the synthetic than on the relatively natural diet, suggesting that the former still lacked factors which the latter contained.

To appreciate the biological significance of running in deprivation it is necessary to realize that it occurs in a behavioral sequence just the reverse of what it is commonly supposed to be. One is used to thinking that an animal first experiences *sensations* of want, then performs appropriate *actions* to satisfy its needs. Such behavior is commonly described as "food-seeking" or "mate-seeking." The futile running of a rat in an activity wheel, however, cannot be characterized in this way; nor can the hunger movements of sleeping animals, or the helpless hunger activity of decorticate animals which make no attempt to take the food placed before them. All these instances show that the primary response to deprivation is activity as such, regardless of sensations or external inducements. If the environment offers the possibility, sensory and cortical mechanisms superimposed upon this primary reaction may guide it into appropriate channels. Otherwise the response remains confined to simple hyperactivity.

In a free environment running should fulfil an important function, since it increases the probability that the animal will encounter what it lacks. In effect it represents a gamble in which the animal's metabolic reserves are staked against the chance of finding its necessities.

The possibility of a successful outcome for the individual, however, is not the only point of this reaction. It probably represents also the behavioral basis of mammalian emigration. This is recognized to be motivated primarily by nutritional need. For the individual emigrant it takes the form of aimless and persistent wandering, without apparent external incentive, and ending almost always in disaster. Its essential biological function is not to rescue the emigrating animal—though this may occur—but to relieve nutritional pressure on the home population. To accomplish this the animal must simply continue to move onward, even though it hasten thereby its own destruction.[15] The running of a deprived animal in an activity wheel provides an accurate model for this behavior.

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[9] Specific deficiency diets were as follows: (*a*) *Thiamin:* the U. S. P. XII thiamin-deficient test ration supplemented with 2 μg. pyridoxine hydrochloride and 10 μg. calcium pantothenate per gram dry food. This was completed on occasion with various amounts of thiamin hydrochloride. (*b*) *Riboflavin:* ration B of Axelrod and Elvehjem (*J. Biol. Chem.*, **140**, 725 (1941)), with its fat content increased to 5 per cent, and supplemented with 10 mgm. mixed tocopherols fed to each animal twice weekly. This was completed when desired with 2 μg. riboflavin per gram of food. (*c*) *Magnesium:* A slight modification of the Mg-low diet of Kruse, *et al.* (*J. Biol. Chem.*, **96**, 519 (1932)), completed when desired with 0.55 per cent $MgSO_4 \cdot 7H_2O$.

[10] Guerrant, N. B., and Dutcher, R. A., *Jour. Nutrition*, **20**, 589 (1940).

[11] Bloomfield, A. L., and Tainter, M. L., *Federation Proc.*, **2**, 75 (1943).

[12] The term *polyneuritis* is used throughout this paper in a restricted sense to mean the stage of thiamin deficiency in which rats exhibit temporary postural disorientation and paralysis after being twirled by the tail (U. S. Pharmacopeia XII, p. 625).

[13] Smith, P. K., and Smith, A. H., *Amer. Jour. Physiol.*, **109**, 99 (1934).

[14] Guerrant, N. B., Dutcher, R. A., and Chornock, F., *Jour. Nutrition*, **17**, 473 (1939).

[15] "The act of mass emigration—is performed by a surplus population. . . . The main incitement—would appear to be want of food. . . and the result in the end is invariably disaster" (Heape, W., *Emigration, Migration and Nomadism*, Heffer and Sons, Cambridge, 1931, p. 21). "It can now be seen that the apparent mystery of where the lemmings are *going to* on their migrations is cleared up when we assume that they are *going away from* the mountains on account of some unfavourable conditions caused by increased density of population" (Elton, C., *Voles, Mice and Lemmings*, Oxford Univ. Press, 1942, p. 215). That man may share to a degree in this response-pattern is suggested by the following account from Herodotus (I, 94) of the invention of athletic games: "In the reign of Atys, son of Manes, there was a great famine throughout all Lydia. The Lydians bore the calamity patiently for some time, but seeing that it did not stop, they set to work to devise remedies for the evil. The device adopted against the famine was to give up one day so entirely to playing as not to feel any want of food, and the next day to eat and stop the games. In this manner they continued for eighteen years. As the affliction lasted and even became more grievous, the king divided the nation in half, and made the two portions draw lots, the one to stay, the other to emigrate from the country."

THE EPIDEMIC CURVE WITH NO ACCESSION OF SUSCEPTIBLES

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In a previous paper[1] we established a higher approximation than $z = z_0 \operatorname{sech}^2 \sqrt{z_0/2}\, T$ to the epidemic curve satisfying Soper's formulation of the problem for such a disease as measles, when recruits to the susceptible population are neglected, namely,

$$T = \frac{1}{6} \log (z/z_0) \pm \frac{1}{3}\sqrt{1 + 18/z_0}\, \tanh^{-1} \sqrt{1 - z/z_0}, \qquad (1)$$

where T is measured from the peak of the epidemic in units of time equal to the "incubation period" and z_0 is the case rate for new infections at the peak measured in terms of m, the number of susceptibles just sufficient to maintain the disease in a steady state provided an adequate supply of recruits were available. As $z = -dx/dT$ by definition, the number of susceptibles may be obtained by integration, namely,

$$x = \text{const.} + \int z dT = \text{const.} + \int z(dT/dz)dz$$

which, after considerable manipulation, turns out to be

$$x = \text{const.} + \frac{1}{6}(z_0 - z) \pm \frac{1}{3}\sqrt{18 + z_0}\, \sqrt{z_0 - z}, \qquad (2)$$

where the positive sign applies before the peak of the case rate and the negative sign after it. From this form it follows that cases measured relative to m must be

$$\text{total cases} = 2\sqrt{2z_0}\sqrt{1 + z_0/18} \qquad (3)$$

which may be compared with the result total cases $= 2\sqrt{2z_0}$ for the first approximation $z = z_0 \operatorname{sech}^2 \sqrt{z_0/2}\, T$. As z_0 is a small number compared to 18, the result (3) is not materially different from that obtained before. The ratio of total cases to the case rate at the peak of the epidemic is therefore[2] approximately

$$\frac{\text{total cases}}{\text{peak case rate}} = 2\sqrt{\frac{2}{z_0}} = \frac{2}{B}, \qquad (4)$$

where B is the value obtained in fitting $z = z_0 \operatorname{sech}^2 BT$ and for clear-cut epidemics of measles seems to range from about 0.20 to about 0.60.

To determine the value of x in (2), it is necessary to find the value of the constant of integration. The fundamental equation

$$\frac{dx}{dT} = x\left.\frac{dx}{dT}\right|_{T-1} \quad \text{or} \quad \left.\frac{d\log x}{dT}\right|_{T+1} = \frac{dx}{dT}, \tag{5}$$

when $A = 0$, can be integrated exactly as

$$\log x\,(T+1) = x(T) - k. \tag{6}$$

To effect a comparison of (6) and (2), it is necessary to determine the constant k. We start by expanding $x(T)$ in series with the assumption that the initial conditions are $T = 0$ when $x = 1$, namely,

$$x = 1 + aT + bT^2 + cT^3 + dT^4 + eT^5. \tag{7}$$

Substituting in (6) we find b, c, d, e, in terms of a; the maximum of the case rate curve comes at the value of T such that $d^2x/dT^2 = 0$ and for that value $z = -dx/dT = z_0$. Thus a and the other coefficients may be found in terms of z_0 and finally since $\log x(0) = \log 1 = 0$, $k = x(-1)$ may be found in terms of z_0. The result is[3]

$$\log x(T+1) = x(T) - 1 - z_0 + \frac{z_0^2}{24} - \frac{z_0^3}{180}. \tag{8}$$

This equation may be used to compute $x(T)$ stepwise from any value for $x(T)$ assumed at any time T. If the time T at which $d^2x/dT^2 = 0$ is substituted in (7) we find the value of x at the peak of the epidemic when $z = z_0$, which is precisely the value of the constant in (2). The result is[4]

$$\text{const.} = 1 + \frac{z_0}{2} - \frac{z_0^2}{24} + \frac{59z_0^3}{3840}. \tag{9}$$

Thus, given any value of z_0, we may compare the approximation (2) with the result of a stepwise calculation from (8).

For example, let us return to the hypothesis that $z_0 = 0.3$. Then the relation between the susceptibles x and the case rate z is

$$x = 1.1467 + \frac{1}{6}(0.3 - z) \pm \frac{1}{3}\sqrt{18.3}\,\sqrt{0.3 - z}. \tag{10}$$

Further we have

$$\log x(T+1) = x(T) - 1.2964, \qquad z(T+1) = x(T+1)\,z(T). \tag{11}$$

Starting from the peak at $T = 0$ we have $x(0) = 1.1467$. Then $x(1) = 0.8609$ and $z(1) = 0.2583$. Thus we may compute table 1.

In the table of values the columns for x and z by (11) give the values that are exact in so far as the constant k may be considered as determined from (8) with neglect of powers of z_0 higher than the third; these columns

TABLE 1

VALUES OF x AND z WHEN $z_0 = 0.3$

T	x BY (11)	z BY (11)	x BY (10)	z BY (1)
0	1.1467	0.3000	1.1467	0.3000
+1	0.8609	0.2583	0.8615	0.2580
−1	1.4333	0.2616	1.4331	0.2614
+2	0.6470	0.1671	0.6479	0.1665
−2	1.6563	0.1825	1.6560	0.1821
+3	0.5223	0.0873	0.5239	0.0868
−3	1.8010	0.1102	1.8004	0.1098
+4	0.4611	0.0403	0.4629	0.0400
−4	1.8847	0.0612	1.8836	0.0610
+8	0.4151	0.0013	0.4172	0.0013
−8	1.9725	0.0044	1.9714	0.0043
$+\infty$	0.4136		0.4157	
$-\infty$	1.9790		1.9777	

represent an integration of the fundamental difference equation regulating the epidemic. The columns for x and z by (10) and by (1) are calculated from the approximation (1) which we developed in our previous paper as a good approximation to the integral of the difference equation and from its integral (2). A comparison of the two pairs of columns will therefore show how good the approximation is.

The exact integral (6) enables one to compute the course of a hypothetical epidemic for comparison with the cruder computations by the Soper or Frost finite equations.[5] If we have $m = 1000$ and $S_0 = 2000$ and introduce one case, which will then generate two cases, we have at the start $x(T + 1) = 1.998$ and $x(T) = 2.000$. Then k in (6) is

$$k = 2.000 - \log 1.998 = 1.30785 \qquad (12)$$

and with this value of k, one may use (6) to follow the subsequent course in each step of which the value of x will be cut down so that $S = 1000x$ is integral. The cases may then be obtained by subtracting successive values of x. Table 2 gives the comparison of this calculation with the previous ones. From the result it is seen that the stepwise calculation departs widely from that of Soper but is very much the same as that of Frost (as modified by introducing the law of small numbers).

As a matter of fact we have the equations

$$C_{t+1} = S_t[1 - e^{-rC_t}], \qquad S_{t+1} = S_t - C_{t+1}$$

and if C be eliminated we have

$$S_{t+1} = S_t\, e^{r[S_t - S_{t-1}]}$$

or $$\log S_{t+1} - rS_t = \log S_t - rS_{t-1};$$

TABLE 2

COURSE OF HYPOTHETICAL EPIDEMIC $C_0 = 1$, $S_0 = 2000$, $r = 0.001$

GENERATION	BY (6) AND (12)	SOPER	FROST
1	2	2	2
2	4	4	4
3	8	8	8
4	16	16	16
5	31	32	32
6	59	62	61
7	107	116	111
8	180	204	186
9	263	317	268
10	308	393	309
11	271	332	267
12	179	171	173
13	94	59	89
14	43	17	40
15	18	5	17
16	8	1	7
17	3	0	3
18	1	..	1
19	1	..	0
20	0	..	..
Total infected	1596	1739	1594
Residual susceptibles	404	261	406

each of these terms must therefore be the same constant and the relation is therefore equivalent to (6). Hence, although the argument which led to the equations based on considering the epidemic to proceed by discrete generations with the elimination of double contacts was very different from that based on a continuous process and embodied in a differential-difference equation, the final results have been seen to be equivalent.[6]

The characteristics of the curve of case rates (epidemic curve) based upon the general exact solution (8) are not easily obtained, but as table 1 indicates that the values derivable by calculation based upon (11) are so nearly the same as those obtained from the approximation (1), it is of interest to give some of the characteristics of (1), which probably will be a sufficient approximation to the corresponding characteristics of the exact curve. The values

$$\nu_i = \int_{-\infty}^{\infty} T^i z dT = \int_{-\infty}^{\infty} T^i z \frac{dT}{dz} dz$$

may be obtained by breaking the integral up into the part from $-\infty$ to z_0 and the part from z_0 to ∞ with the appropriate expressions taken from (1). Certain definite integrals are involved, of which some may be evaluated in terms of known constants and others may be had by computation. From the values of ν_i the moments may be computed.

The mean is

$$\frac{1}{3}\log 2 - \frac{1}{2} = -0.26895,$$

$$\mu_2 = \frac{\pi^2}{6z_0} + 0.07070, \qquad \mu_3 = -\frac{1.2020}{z_0} - 0.10424.$$

From this it is seen that the mean (referred to the mode) is negative and independent of z_0. Except for the small term 0.07070, the square of the standard deviation is the same as that for the first approximation $z = z_0 \operatorname{sech}^2 \sqrt{z_0/2}\,T$. The negative value of μ_3 shows that the skewness is negative (the epidemic curve rises more slowly than it falls) and if the small additive terms be neglected it is $\kappa = \mu_3/\mu_2^{3/2} = -0.57 z_0^{1/2}$.

The skewness is not only negative but large. Brownlee noted that observed epidemics were remarkably symmetrical and that such skewness as they had was much more likely to be positive than negative; he inferred therefrom that the want of persons liable to infection is not the cause of the decay of the epidemic.[7] Even though the want of persons liable to infection is not a sufficient cause to explain the slope of epidemic curves, it does seem that for immunizing diseases like measles it must be a necessary cause in any ultimate explanation. It is therefore of interest to have (1) an exact integral of the differential-difference equation which regulates that part of the phenomenon that derives from the law of mass action and (2) a satisfactorily close approximation to that integral in manageable form.

[1] These PROCEEDINGS, **30**, 37–44 (1944).

[2] If we take the four epidemics used in figure 1 of the previous paper, we have:

EPIDEMIC	TOTAL CASES	PEAK CASE RATE	RATIO	$2/B$
Vancouver, B. C., 1931–1932	3898	1150	3.4	3.6
Berkeley, Calif., 1938–1939	3073	625	4.9	4.8
Providence, R. I., 1934–1935	5267	1000	5.3	4.8
Oakland, Calif., 1933–1934	4889	625	7.8	8.0

The Vancouver epidemic was extremely explosive. The values of the peak case rate are roughly estimated. The agreement between the last two columns is reasonably satisfactory.

[3] The value of k goes only up to terms in z_0^3 although x was expanded to T^5. There is "loss of places" in an algebraic sense in carrying out the work.

[4] In these PROCEEDINGS, **29**, 43–48 (1943), the expression $z = z_0 \operatorname{sech}^2 \sqrt{z_0/2}\,T$, or its equivalent, was integrated to obtain $x = 1 - \sqrt{2z_0}\tanh \sqrt{z_0/2}\,T$, or equivalent, on the assumption that at the peak when $T = 0$ the value of x would be 1. This was in fact one of Soper's assumptions in that he took, in our notation, $1 + du/dT = x$ as one of the starting points of his developments, and this makes $x = 1$ when u is a maximum. It may be that to the order of approximation which Soper uses, it is satisfactory to take $x = 1$ at maximum; but if we go back to the equation from which he starts, viz., $z(T) = x(T)z(T - 1)$ we find that

$$x = \frac{z}{z - \dfrac{dz}{dT} + \dfrac{1}{2}\dfrac{d^2z}{dT^2}} = 1 + \frac{1}{z}\frac{dz}{dT} - \frac{1}{2z}\frac{d^2z}{dT^2} + \frac{1}{z^2}\left(\frac{dz}{dT}\right)^2$$

so that if $dz/dT = 0$ when $z = z_0$ we obtain

$$x = 1 - \frac{1}{2z_0}\frac{d^2z}{dT^2}\bigg|_0 \text{ and from } d^2u\,\frac{d^2u}{dT^2} = -e^u = -z$$

to which he comes, after his various approximations, we find $x = 1 + z_0/2$ as presumably a better result, and this checks with (9). This would make

$$x = 1 + \frac{1}{2}z_0 - \sqrt{2z_0}\tanh\sqrt{z_0/2}\,T$$

with the initial and final values of x, $x_0 = 1 + \frac{1}{2}z_0 + \sqrt{2z_0}$, $x_E = 1 + \frac{1}{2}z_0 - \sqrt{2z_0}$ and with the relationship $x_0 + x_E = 2 + z_0$ instead of $x_0 + x_E = 2$.

[5] These PROCEEDINGS, **28**, 361–367 (1942).

[6] The slight numerical differences between the second and fourth columns in table 2 are due to differences in the rejection of fractional cases.

[7] Brownlee, J., *Proc. Roy. Soc. Med., London*, **2**, Pt. 2, 243–258 (1909). See page 244. This is quoted (on page 206) by Sir Ronald Ross, *Proc. Roy. Soc. London*, **A92**, 204–230 (1915), and by C. O. Stallybrass on p. 510 of his *Principles of Epidemiology*, Chap. 14, 1931.

INTEGRAL FORMULAS FOR THE CHARACTERISTIC CLASSES OF SPHERE BUNDLES

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The notion of sphere bundles[1] of H. Whitney has proved to be very fruitful in the applications of topology. So far in this theory an important rôle has been played by a class of topological invariants in the base space, called the characteristic cohomology classes and which can be regarded as generalizations of the Euler-Poincaré characteristic. By assuming the base space to be a differentiable manifold with a Riemannian metric, on which the bundles of vectors and p-vectors are defined in a natural way, we shall give in this note various relations which exist between these topological invariants and certain differential invariants of the Riemannian manifold. In fact, the relations are such that the former are expressed as integrals of the latter. In the formulae the generalized Gauss-Bonnet formula due to Allendoerfer and Weil[2] is included as a particular case.

1. At the basis of our considerations is a closed orientable n-dimensional Riemannian manifold of class C^m, $m \geqq 4$, which we denote by R^n. In R^n

we consider the frames $P\,e_1 \ldots e_n$, each of which consists of a point P and n mutually perpendicular unit vectors $e_1, \ldots, e_n$ through P, arranged in an order coherent with the orientation of R^n. Using the notations of E. Cartan,[3] the parallelism of Levi-Civita is defined by equations of the form

$$dP = \omega_i e_i, \qquad de_i = \omega_{ij} e_j, \qquad \omega_{ij} + \omega_{ji} = 0, \tag{1}$$

where the ω's are Pfaffian forms. In terms of these Pfaffian forms the equations of structure of the space are

$$d\omega_i = \omega_j \omega_{ji}, \qquad d\omega_{ij} = -\omega_{ik}\omega_{jk} + \Omega_{ij}, \tag{2}$$

and the identities of Bianchi are

$$\omega_j \Omega_{ji} = 0, \qquad d\Omega_{ij} = -\omega_{ik}\Omega_{jk} + \omega_{jk}\Omega_{ik}. \tag{3}$$

It is to be mentioned that Ω_{ij} are exterior quadratic differential forms which give the curvature properties of R^n.

Consider now the tangent sphere bundle of R^n in the sense of Whitney and, in particular, the problem of defining over R^n a field of p-vectors, $1 \leqq p \leqq n$, where by a p-vector is meant an ordered set of p vectors (and not the figure determined by the Grassmannian coördinates). As is well known, the characteristic classes arise from the points of R^n at which the p vectors are linearly dependent. Topologically there is no loss of generality to assume the p vectors, when linearly independent, to be mutually perpendicular unit vectors.

We are thus led to consider in R^n the figures formed by a point P and an ordered set of p mutually perpendicular unit vectors through P. With P fixed, all these figures form, according to a natural topology, a finite manifold $V_{n,p}$ of dimension $p(2n - p - 1)/2$. The Betti group $B^{n-p}(V_{n,p})$ of dimension $n - p$ (with integer coefficients) of $V_{n,p}$ is an infinite cyclic group or a cyclic group of order two, according as one of the following conditions is satisfied or not: $p = 1$ or $n - p$ is even. We consider the sphere bundle $\mathfrak{S}(p)$ with R^n as the base space and the $V_{n,p}$ as fibres and proceed to study the relations between the characteristic classes arising therefrom and certain differential invariants of R^n.

2. With our problem formulated in this way, it is convenient to adopt the language of the method of moving frames. To a figure of the above description we attach a frame $Pe_1 \ldots e_n$ such that P is the origin and $e_{n-p+1}, \ldots, e_n$ the p vectors of the figure in question. With the intervention of the family of frames thus attached, Pfaffian forms ω_i, ω_{ij} and differential invariants can be constructed from (1, 2). From them it is possible to construct exterior differential forms of higher degree. Whether they belong to $\mathfrak{S}(p)$ or not can be decided by the following simple criterion: *A differential form* Π *belongs to* $\mathfrak{S}(p)$ *if and only if its exterior derivative* $d\Pi$ *is a form in* ω_i, ω_{iA} $A = n - p + 1, \ldots, n$, *only*. A differential form belonging to $\mathfrak{S}(p)$ is called intrinsic.

3. For our considerations we shall suppose either $p = 1$ or $n - p$ be even. The essential step consists of establishing a formula for differential forms which we shall reproduce here for the case that $n - p$ is even. We consider the following intrinsic differential forms

$$(2k;\ h_1, \ldots, h_p) = \epsilon_{\alpha_1 \ldots \alpha_{n-p}} \Omega_{\alpha_1\alpha_2} \ldots \Omega_{\alpha_{2k-1}\alpha_{2k}} \omega_{\alpha_{2k+1}} \ldots \omega_{\alpha_{2k+h_1}} \ldots \omega_{\alpha_{2k+h_1+\cdots+h_{p-1}+1}} \ldots \omega_{\alpha_{2k+h_1+\cdots+h_p}} \quad (4)$$

where each index α runs from 1 to $n - p = 2k + h_1 + \ldots + h_p$ and where $\epsilon_{\alpha_1 \ldots \alpha_{n-p}}$ denotes the Kronecker symbol which is $+1$ or -1 according as $\epsilon_{\alpha_1, \ldots, \alpha_{n-p}}$ form an even or odd permutation of $1, \ldots, n - p$, and is otherwise zero. We put also

$$\Phi_k = \sum \frac{2.4 \ldots (n - p - 2k)}{(2.4 \ldots 2q_1) \ldots (2.4 \ldots 2q_p)} (2k;\ 2q_1, \ldots, 2q_p) \quad (5)$$

where the summation is extended over all partitions of $\frac{1}{2}(n - p) - k$ as a sum of $q_1, \ldots, q_p$. Further let

$$a_k = (-1)^k \frac{(n - p - 2k + 2) \ldots (n - p)}{2.4 \ldots 2k}, \quad k \geqq 1, \quad (6)$$

$$a_0 = 1.$$

Then we have the formula

$$d\left\{\sum_{k=0}^{1/2(n-p)} a_k \Phi_k\right\} = 0. \quad (7)$$

The proof of this formula can be carried out in a purely algebraic way.

4. Suppose we consider the p-vectors having the same origin, i.e., those belonging to the same $V_{n,p}$. Under this condition we have evidently

$$\omega_1 = \ldots = \omega_n = 0,$$

and formula (7) becomes

$$d\Phi_0 = 0$$

This shows that Φ_0 is a closed intrinsic form in $V_{n,p}$. Noticing that $B^{n-p}(V_{n,p})$ is an infinite cyclic group, it follows from the well-known results of de Rham[4] that the value of the integral

$$\int_Z \Phi_0$$

where Z is an $(n - p)$-dimensional cycle of $V_{n,p}$, will give the homology class to which Z belongs.

The theory of p-fields in R^n leads, when $n - p$ is even, to an integral characteristic class W^{n-p+1}. To derive from it a numerical topological invariant we consider the scalar product $W^{n-p+1}M^{n-p+1}$, where M^{n-p+1} is a submanifold of dimension $n - p + 1$ in R^n, closed or with boundary. When M^{n-p+1} is closed, we derive from (7) and the above remark that

$$W^{n-p+1}.M^{n-p+1} = 0,$$

which corresponds to the known fact that W^{n-p+1} is of finite order.

If, however, M^{n-p+1} has a boundary B, we shall get in this way a formula which links the integral of a differential invariant over B with a topological invariant of M^{n-p+1}. For definiteness we suppose $p = 2$ (so that n is even). B is of dimension $n - 2$ and we suppose for simplicity that B possesses a tangent $(n - 2)$-plane at each of its points. Both M^{n-1} and B are supposed to be orientable, so that M^{n-1} is two-sided in R^n and B two-sided in M^{n-1}. At each point of B we take as e_n the outside (or inside) normal of M^{n-1} and as e_{n-1} the outside (or inside) normal of B in the tangent hyperplane of M^{n-1}. We have thus defined over B a field $\mathfrak{F}$ of two vectors and the form

$$\Pi = \sum_{k=0}^{1/2(n-2)} a_k \Phi_k,$$

being intrinsic in $\mathfrak{S}(2)$, is defined on B. Then we have

$$\int_B \Pi = c.M^{n-1}.LL^{n-1}$$

where c is a numerical constant. This formula is the analog of the Gauss-Bonnet formula for a polyhedron. The corresponding formulation for a general p is without difficulty.

5. As we have shown in another occasion,[5] these discussions lead for $p = 1$ to a proof of the Gauss-Bonnet formula by which it is not necessary to imbed the cells of R^n in a Euclidean space of sufficiently high dimension. There the formula corresponding to (7) is of the form

$$d\Pi = \Omega,$$

where $\Omega = 0$ when n is odd. The differential form Π, of degree $n - 1$, is defined in the space of vectors and has some interest in itself. It is defined, whenever an orientable submanifold M^r, of dimension r, is given in R^n. For, supposing that M^r has a tangent r-plane at each of its points, we attach to a point of M^r all the unit normal vectors at that point if $r \neq n - 1$ and the outside (or inside) unit normal vector at that point if $r = n - 1$. In this way the set of unit vectors attached to the points of M^r forms, according to the natural topology, a manifold of dimension $n - 1$. This process can also be carried out for M^r which ceases to have tangent r-

planes at certain points. Then we attach to each of these points the unit normal vectors belonging to the corresponding dual angle. By such a convention the differential form Π is defined over M^r and generalizes the notion of the element of geodesic curvature for $n = 1$. We can prove that, if r is even,

$$\int_{M^r} \Pi = c\chi(M^r),$$

where $\chi(M^r)$ stands for the Euler-Poincaré characteristic of M^r and c is a constant. The proof of this formula is somewhat complicated.

6. It is evident that the same considerations can be carried out for the normal sphere bundles of a submanifold imbedded in a differentiable manifold. To avoid duplication we shall not give the results here. We only mention that the corresponding differential invariants are those which give the so-called Gaussian torsion of the submanifold.

7. Finally, we wish to make the following remark. The process employed here bears a close resemblance to that used by Gysin[6] in his work on the homology theory of fibre spaces. Our differential forms take the place of cochains and we have taken the group of real numbers as the coefficient group of the homology theory. While we thus lose such topological invariants like torsion, we are compensated by the fact that the differential forms are intrinsic in the sense of differential geometry and have thus arrived at results which connect differential invariants with topological invariants.

[1] Whitney, H., "On the Topology of Differentiable Manifolds," *Lectures in Topology* Michigan, 1941, pp. 101–141. Also: Stiefel, E., "Richtungsfelder und Fernparallelismus in n-dimensionalen Mannigfaltigkeiten," *Comm. Math. Helv.*, **8**, 305–353 (1936).

[2] Allendoerfer, C. B., and Weil, André, "The Gauss-Bonnet Theorem for Riemannian Polyhedra," *Trans. Amer. Math. Soc.*, **53**, 101–129 (1943).

[3] Cartan, E., *Leçons sur la géometrie des espaces de Riemann*, Paris, 1928.

[4] de Rahm, G., "Sur l'analysis situs des variétés a n dimensions," *J. Math. Pures Appl.*, **10** (9), 115–200 (1931).

[5] Chern, S., "A Simple Intrinsic Proof of the Gauss-Bonnet Formula for Closed Riemannian Manifolds," to appear in *Annals of Math.*

[6] Gysin, W., "Zur Homologietheorie der Abbildungen und Faserungen von Mannigfaltigkeiten," *Comm. Math. Helv.*, **14**, 61–122 (1941–1942).

ON GROUPS OF AUTOMORPHISM OF LIE GROUPS

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Let $\mathfrak{G}$ be a simply connected Lie group, and let $\mathfrak{g}$ be the Lie algebra of $\mathfrak{G}$. Then the group of automorphisms $\mathfrak{A}$ of $\mathfrak{G}$ is isomorphic to a linear group $\mathfrak{A}^*$ operating on the vector space $\mathfrak{g}$. The infinitesimal transformations of $\mathfrak{A}^*$ are the linear mappings D of $\mathfrak{g}$ into itself which have the property that

$$D([X, Y]) = [DX, Y] + [X, DY] \tag{1}$$

holds for any X and Y in $\mathfrak{g}$. These mappings are called the *derivations* of the Lie algebra $\mathfrak{g}$. They form a Lie algebra which is called the *algebra of derivations* of $\mathfrak{g}$. We shall denote this algebra by $\mathfrak{D}(\mathfrak{g})$.

The inner automorphisms of $\mathfrak{G}$ form a distinguished subgroup $\mathfrak{A}_0$ of $\mathfrak{A}$. The above-mentioned isomorphism of $\mathfrak{A}$ with $\mathfrak{A}^*$ maps $\mathfrak{A}_0$ onto the adjoint group $\mathfrak{A}_0^*$ of $\mathfrak{G}$. The infinitesimal transformations of $\mathfrak{A}_0^*$ are the derivations D_Z of $\mathfrak{g}$ which can be defined by $D_Z(X) = [X, Z]$ (for some fixed $Z \in \mathfrak{g}$). They are called the *inner derivations* of $\mathfrak{g}$. They form an ideal in $\mathfrak{D}(\mathfrak{g})$, and this ideal is isomorphic to the factor algebra $\mathfrak{g}/\mathfrak{z}$, where $\mathfrak{z}$ is the center of $\mathfrak{g}$. We say that $\mathfrak{g}$ is a *complete algebra* when every derivation of $\mathfrak{g}$ is an inner derivation. Cartan has proved that every semi-simple Lie algebra is complete. Zassenhaus has proved that, if $\mathfrak{g}$ is a Lie algebra whose center reduces to $\{0\}$ and which coincides with its derived algebra, then $\mathfrak{D}(\mathfrak{g})$ is a complete algebra.

Starting with an arbitrary Lie algebra $\mathfrak{g}$, we can define inductively a sequence of Lie algebras $\mathfrak{D}_n(\mathfrak{g})$ in the following way: $\mathfrak{D}_0(\mathfrak{g}) = \mathfrak{g}$; if $\mathfrak{D}_n(\mathfrak{g})$ has been defined, $\mathfrak{D}_{n+1}(\mathfrak{g})$ is $\mathfrak{D}(\mathfrak{D}_n(\mathfrak{g}))$. The object of this note is to announce the following result:

THEOREM. *Let $\mathfrak{g}$ be a Lie algebra over a field of characteristic 0, and assume that the center of $\mathfrak{g}$ contains only 0. Then there exists an index n such that $\mathfrak{D}_n(\mathfrak{g})$ is complete, and therefore all algebras $\mathfrak{D}_n(\mathfrak{g})$ are isomorphic with each other from a certain rank on.*

Only broad outlines of the proof can be given here. It is clearly sufficient to prove the theorem for Lie algebras over the field of complex numbers. Under this condition, $\mathfrak{D}(\mathfrak{g})$ is the Lie algebra of an *algebraic* linear group. From this fact, one can deduce that $\mathfrak{D}(\mathfrak{g})$ may be represented as the direct sum of three subalgebras $\mathfrak{f}$, $\mathfrak{a}$ and $\mathfrak{n}$ with the following properties: $\mathfrak{f}$ is semi-simple; $\mathfrak{a}$ is abelian; $\mathfrak{n}$ is the maximal nilpotent ideal; the matrices in $\mathfrak{a}$ can be simultaneously reduced to the diagonal form, and they commute with the elements of $\mathfrak{f}$.

Let $\mathfrak{n}_0$ be the subalgebra of $\mathfrak{n}$ generated by the elements of the form $[A,$

$N]$ or $[A, S]$, with $A \epsilon \mathfrak{a}$, $N \epsilon \mathfrak{n}$ $S \epsilon \mathfrak{f}$, and set $\mathfrak{d}_0 = \mathfrak{f} + \mathfrak{a} + \mathfrak{n}_0$. Then $\mathfrak{d}_0$ is an ideal in $\mathfrak{D}(\mathfrak{g})$, and the centralisator of $\mathfrak{d}_0$ in $\mathfrak{D}(\mathfrak{g})$ contains only O. Let $J(\mathfrak{d}_0)$ be the ideal formed in $\mathfrak{D}(\mathfrak{d}_0)$ by the inner derivations of $\mathfrak{d}_0$. By a derivation of $J(\mathfrak{d}_0)$ into $\mathfrak{D}(\mathfrak{d}_0)$ is meant a linear mapping of $J(\mathfrak{d}_0)$ into $\mathfrak{D}(\mathfrak{d}_0)$ which satisfies condition (1) above for any X and Y in $J(\mathfrak{d}_0)$. It can be proved that any such derivation maps $J(\mathfrak{d}_0)$ into itself and is therefore induced by some inner derivation of $\mathfrak{D}(\mathfrak{d}_0)$. From this, one can deduce that, $\mathfrak{h}$ being any subalgebra of $\mathfrak{D}(\mathfrak{d}_0)$ containing $J(\mathfrak{d}_0)$, $\mathfrak{D}(\mathfrak{h})$ is isomorphic to some subalgebra of $\mathfrak{D}(\mathfrak{d}_0)$ containing $\mathfrak{h}$, from which it follows immediately that $\mathfrak{D}_n(\mathfrak{h})$ is complete for sufficiently large n. On the other hand, one can see that $\mathfrak{D}(\mathfrak{g})$ itself is isomorphic to a subalgebra of $\mathfrak{D}(\mathfrak{d}_0)$ containing $J(\mathfrak{d}_0)$, whence the result to be proved.

The restriction that the center of $\mathfrak{g}$ be $\{O\}$ cannot be removed from the statement of our Theorem, because examples show that the following two situations may arise by proper choices of $\mathfrak{g}$: I, the dimension of $\mathfrak{D}_n(\mathfrak{g})$ increases indefinitely with n; II, the algebras $\mathfrak{D}_n(\mathfrak{g})$ are all isomorphic with each other for $n \geqslant 1$, but none of them is complete. Nevertheless, there are large classes of nilpotent Lie algebras $\mathfrak{g}$ for which $\mathfrak{D}_n(\mathfrak{g})$ ultimately becomes complete. Let, for instance, L_q be the free Lie ring with q generators, and define $L_q^{(r)}$ by induction on r in the following way: $L_q^{(0)} = L_q$; $L_q^{(r+1)}$ is the set of linear combinations of elements of the form $[X, Y]$, with $X \epsilon L_q$, $Y \epsilon L_q^{(r)}$. Then $L_q^{(r)}$ is an ideal in L_q and $L_q/L_q^{(r)}$ is a nilpotent algebra $\mathfrak{g}(q, r)$. It can be proved that $\mathfrak{D}_2(\mathfrak{g}(q, r))$ is complete provided $q > 1, r > 1$.

On the other hand, it follows immediately from our theorem that, if $\mathfrak{g}$ coincides with its derived algebra, then $\mathfrak{D}_n(\mathfrak{g})$ is complete for n sufficiently large. In fact, it is easy to see that the center of $\mathfrak{D}_1(\mathfrak{g})$ contains only O.

GROUPS CONTAINING LESS THAN TWENTY-EIGHT NON-INVARIANT OPERATORS

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In two preceding numbers of these Proceedings (**30**, 25–28 (1944), **30**, 114–117 (1944)) various methods were noted for the determination of the possible groups when the number of the non-invariant operators is given. The object of the present article is to give a few additional such methods and to illustrate these methods by means of the determination of all the groups which separately involve less than twenty-eight non-invariant op-

erators. It may be noted that in the early determination of permutation groups and abstract groups A. L. Cauchy (1789–1857), A. Cayley (1821–1895) and others proceeded similarly with a view to introducing their readers to the subjects which were later extensively developed by others by means of improved methods.

A useful theorem in the said determination may be expressed as follows: *If a group of order $m + n$ contains n invariant operators and m non-invariant ones then n is a divisor of m and cannot exceed $m/2$.* This theorem is evident but it seems desirable to state it explicitly in order to simplify its applications in what follows. It may be desirable to recall here the theorem that if a group contains a prime number p of non-invariant operators and all of its other operators are invariant then it contains exactly one invariant operator and therefore its order is $p + 1$. As illustrative examples we note the non-cyclic group of order 6, the tetrahedral group, and the simple group of order 60, which is the simple group of smallest composite order.

Since all the groups which separately contain less than twenty-four non-invariant operators can readily be determined by means of the noted theorems we proceed to consider here, with some details, all the groups which contain separately exactly twenty-four non-invariant operators. It is obvious that the order of such a group is either 27 or 32, and if it is 27 the group must be one of the two non-abelian groups of this order. It is therefore only necessary to consider groups of order 32 in determining the remaining groups which separately contain twenty-four non-invariant operators. Moreover, the only groups of order 32 which require consideration in this connection are those which contain separately just eight invariant operators and hence have for their central quotient group the non-cyclic group of order 4, since the central quotient group of a group cannot be cyclic.

It therefore results that all the groups of order 32 which we need to consider here contain three abelian subgroups of index 2 whose cross-cut is the central of the group G. Moreover, the commutator subgroup of each of these groups is in the central of G and is of order 2. It will be convenient to consider separately the possible groups when the central is a given group beginning with the case when this central is cyclic. In this case G may contain a cyclic group of order 16 and if it has this property two of the three abelian subgroups of index 2 in G are cyclic while the third is of type 3, 1. There is obviously one and only one such G. When the central of G is cyclic but G does not involve any operator of order 16 the three abelian subgroups of G are all of type 3, 1 and there is clearly one and only one such G. We have now determined the two possible groups when the central of G is cyclic.

The central of G in the remaining groups is either of type 2, 1 or of type

1, 1, 1. We begin with the former of these two possible cases. In this case the commutator subgroup of G is either the square of the operators of order 4 contained in the central or it is one of the other two operators of order 2 in the central. We begin with the case when the central is of type 2, 1 and when a commutator is the square of an operator of order 4 contained in the central. If such a G contains operators of order 8 there are two possible groups. In both of these groups two of the three abelian subgroups of index 2 are of type 3, 1. In one of them the third subgroup of this index is of type 2, 1, 1 while in the other it is of type 2, 2. To complete the determination of the groups which involve operators of order 8 we have yet to consider the case when the commutator of order 2 is not the square of an operator of order 4 contained in the central of G.

In this case there are again two groups in which two of the three abelian subgroups of index 2 are of type 3, 1. In one of these the third abelian subgroup of index 2 is again of type 2, 1, 1 while in the other it is of type 2, 2. In these groups the cyclic subgroups of order 8 are non-invariant while in each of the preceding four groups the corresponding cyclic subgroups are invariant. It remains to determine the groups which involve no operator whose order exceeds 4 and in which the central is either of type 2, 1 or of type 1, 1, 1. If in the former case G involves an abelian subgroup of index 2 and of type 2, 2 it may first be assumed that the commutator subgroup of G is generated by an operator of order 4 contained in the central of G. In this case the remaining operators of G must include an operator of order 2. If this were not the case all the remaining operators of G would be of order 4 and could not have for their squares a commutator of G.

If s_1 is such an operator and s_2 is an operator of order 4 in the given subgroup of type 2, 2 which is not in the central then $(s_1s_2)^2 = s_1^2s_2^2$ into the commutator of order 2 in G. It therefore results that G would involve an operator of order 2 which is not contained in the given subgroup of type 2, 2. There is clearly one and only one such group. One of its three abelian subgroups of index 2 is of type 2, 1, 1 while the other two are both of type 2, 2. When the commutator of order 2 in G is not the square of an operator of order 4 in the central of G but G still involves an abelian subgroup of type 2, 2 there are two additional groups. In one of these all of the remaining operators of G are of order 4 while in the other only one-half of these operators have this property. We have now considered all the groups which contain no operator of order 8 and in which one of the abelian subgroups of index 2 is of type 2, 2.

When G contains a central of type 2, 1 but no abelian subgroup of type 2, 2 and no operator of order 8 its three abelian subgroups of index 2 are all of type 2, 1, 1 and the commutator subgroup is generated by an operator of order 4 contained in the central of G. There is one and only one such group. It remains to consider the case when the central is of type 1, 1, 1.

In this case at least one of the abelian subgroups of index 2 in G must be of type 2, 1, 1. If the commutator subgroup is generated by an operator of order 4 in this subgroup of index 2 all the operators of this subgroup are transformed into their inverses and there are three such distinct groups. In one of these all of the remaining operators are of order 2; in another all of these operators have the same square as the operators of order 4 in the given subgroup of index 2, while in the third all of these operators have for their squares a different operator of order 2 contained in this subgroup.

Finally, when the commutator subgroup of G is a different subgroup of order 2 contained in the central there are two additional groups. One of these contains operators of order 2 which are not found in the given subgroup of index 2 while in the other all of these remaining operators are of order 4. We have now determined the seventeen groups which separately contain twenty-four non-invariant operators while all of their other operators are invariant. Two of these groups are of order 27 while each of the remaining fifteen groups is of order 32. These fifteen groups could also be determined by means of the fifty-one known groups of order 32, but the direct determination by means of the fact that each of them contains exactly eight invariant operators seems to be an easier method and illustrates the advantage of considering groups from the standpoint of the number of their non-invariant operators.

If a group contains exactly twenty-five non-invariant operators it may be the dihedral group of order 26 or the direct product of the group of order 5 and the non-abelian group of order 6. As it could obviously be no other group it is a very simple problem to determine all the groups which separately contain exactly twenty-five non-invariant operators. There are also just two groups which separately contain exactly twenty-six non-invariant operators. Both of these groups contain the cyclic group of order 14 and transform all of its operators into their inverses. In one of these groups all of the remaining operators are of order 2 while in the other all of these operators are of order 4 and have a common square. Hence the possible groups which separately contain twenty-six non-invariant operators are also very elementary.

When a group contains exactly twenty-seven non-invariant operators it may be the direct product of the dihedral group of order 10 and the group of order 3. It could not be another group of order 30 since every group of this order contains an invariant subgroup of order 5. Its order could not exceed 36 since the order of a group cannot exceed the number of its non-invariant operators multiplied by 4/3. Its order could not be 36 since its central would then be of order 9 and hence its commutator subgroup could not be of order 2 as it must be in every group of order g in which the number of the non-invariant operators is $3\ g/4$. Hence it results that all the groups which contain less than twenty-eight non-invariant operators have

been determined. When the number of the non-invariant operators of a group is twenty-eight the group may contain a central of order 4 and be of order 32. The number of possible groups in this case seems to be large.

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*A GROUP-THEORETIC CHARACTERIZATION OF THE GENERAL PROJECTIVE COLLINEATION GROUP**

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The success which investigators in the nineteenth century achieved in the study of geometry through the application of group-theoretic ideas may be credited to the celebrated Erlanger Program of Klein. Briefly stated, the main idea of this Program was to consider geometry as a body of invariant theory of transformation groups. The problem immediately arose as to how any given geometry could be generated in this way. To answer such a question one could assume an abstract group and by introducing suitable definitions try to set up a geometry of the desired type. This has been done for plane Euclidean geometry by Thomsen[1] and non-Euclidean geometries by Hjelmslev.[2] The purpose of the present paper is to summarize a solution of the problem in the case of projective geometry.

We shall start with an abstract group and shall define certain transformations in it by means of its inner automorphisms. These inner automorphisms are used to define a projective geometry which has a projective collineation group isomorphic to the original abstract group. As a corollary, we obtain a very simple representation of the complete group of automorphisms of the projective collineation group, without having recourse to coördinates. By a simple specialization of the projective group one can also obtain a representation of Euclidean geometry. We consider here mainly the two-dimensional case; extension to n-dimensions, while by no means immediate, is not difficult.

Let us denote the normalizer of the subgroup P of an abstract group G by the notation $N(P)$. Two subgroups A and B of G will be said to be *strongly* conjugate in G if $B = g^{-1}Ag$, where g can be chosen to be of finite order. We state the following axioms.

AXIOM 1. *Let G be a group generated by two complete sets of strongly conjugate subgroups $\{P\}$ and $\{L\}$ such that the only element common to all subgroups of both sets is the identity.*

DEFINITION: *A subgroup of class $\{P\}$ will be called a point, one of class $\{L\}$ a line.*

AXIOM 2. *If T is a point or line $NN(T) = N(T)$.*

AXIOM 3. *If $A \subseteq N(D)$, there exist two other subgroups B, C conjugate to A and such that $(B, C) \subseteq N(D)$. Of the two groups A and D, one is of class $\{P\}$ and the other of class $\{L\}$.*

AXIOM 4. *(a) For any distinct points P and Q, $(P, Q) = N(L)$ for some line L. (b) For any distinct lines L and M, $(L, M) = N(P)$ for some point P.*

DEFINITION: *A point P is said to be on a line L if $P \subseteq N(L)$. A line L is on a point P if $L \subseteq N(P)$. This symmetry of the relation on has to be proved.*

AXIOM 5. *If A, B, C are three distinct points (lines) on the line (point) D, then $N(A) \cap N(B) \cap N(C) = D$.*

AXIOM 6. *Let P and L be any point and line, respectively. Then*

$$G = N(L) + \sum N(L)g,$$

and either $P \subseteq N(L)$ or for each coset $N(L)g$ such that P not-$\subset g^{-1}N(L)g$, the set $P \cap N(L)g$ is non-null.

Remarks Concerning the Axioms.—As they stand the axioms are not sufficient to define projective geometry, but this can be accomplished by strengthening Axiom 6. The Axioms are consistent since they can be shown to hold in any finite projective geometry. By means of examples, each of Axioms 5, 4, 3, and 1 and 2 together can be shown to be independent of the remaining ones. In Axiom 1 it was found necessary to restrict our sets $\{P\}$ and $\{L\}$ by the condition of "strong" conjugacy. The reason for this is that we are not limiting ourselves to finite groups. It is possible to give examples of infinite groups from which an infinite sequence of subgroups $A_1, A_2, \ldots A_n, \ldots$ can be chosen, such that A_i is conjugate to A_1 but for which $A_i \subset A_{i+1}$ strictly. The use of strong conjugacy eliminates this possibility for the groups $\{P\}$ and $\{L\}$.

That the points P and the lines L form a projective geometry is proved by the following sequence of theorems and definitions:

A point is on a line if and only if the line is on the point.

The group of a point or line is uniquely determined by its normalizer.

Through two points there passes one and only one line, and, dually, on two lines there is only one point.

The condition that points A, B, C, are collinear is that $C \subseteq (A, B)$, and this is equivalent to the condition $A \subseteq (B, C)$ or $B \subseteq (A, C)$.

Any element of G is defined to be a projective collineation. If h is an element of G, then h is said to carry the point A into the point B if $h^{-1}A\,h \subseteq B$.

Any projective collineation carries collinear points into collinear points and concurrent lines into concurrent lines.

A projective collineation t for which $t^{-1}P\,t \subseteq P$ is said to keep the point P fixed. The set of all projective collineations which keep P fixed form the

group $N(P)$ and dually for lines. The group P consists of all projective collineations, each of which keeps every line through P fixed and dually. Perspectivity is defined by means of Axiom 6. We show that corresponding to any two lines L_1 and L_2 and any point O not on either line, there exist collineations t which keep O fixed and which carry L_1 into L_2. The class of all such elements t, is called *perspectivity* of the line L_1 on L_2 from the center O. Dually, perspectivity from a line may be defined.

It follows now that a necessary and sufficient condition for a projectivity t between two lines L_1 and L_2 to be a perspectivity is that t keep fixed the common point of L_1 and L_2. This is equivalent[3] to Pappus' Theorem from which Desargues' Theorem follows in the plane. By sufficiently weakening our axioms one could obtain a geometry for which Desargues' Theorem is not valid but for which the weaker condition, namely, the unique determination of the fourth harmonic point, holds. Such geometries have, in fact, been considered from the geometric point of view, but a study of their groups would also be of interest.

If A, B, C, D and A', B', C', D' are two sets of four points, no three of which in either set are collinear, there exists at most one transformation t of G which carries $A \rightarrow A'$, $B \rightarrow B'$, $C \rightarrow C'$, $D \rightarrow D'$. The actual existence of such an element t cannot be proved until Axiom 6 is replaced by a slightly stronger one.

Let L be any line and P be any point. Let M and N be two lines concurrent with L neither of which passes through P. There is at most one element α of G such that α is in $(P \bigcap L)$ and α: $M \rightarrow N$.

AXIOM 6′: *The element α, described in the above theorem, exists. Axiom 6′ implies its dual and also implies Axiom 6. From Axiom 6′ it follows that there exists exactly one projective collineation which carries one set of four points, no three of which are collinear, into another set.*

The group G and the group of projective collineations of points and lines of G (i.e., its group of inner automorphisms) are isomorphic.

The Automorphisms of G.—The characterization of the projective collineation group we have just given is of a form which makes the study of its complete automorphism group extremely simple.[4] For this it is unnecessary to introduce coördinates. The study is carried out in two parts corresponding to the cases of odd and even dimensional spaces. The reason for this separation is that in a projective space of odd dimensionality there may exist collineations of period 2 without fixed points, and this slightly complicates matters. The special cases of projective spaces without collineations of period 2 (i.e., spaces whose coördinate fields are of characteristic 2) also have to be treated separately. We shall describe briefly the process for the two-dimensional case. The sequence of theorems is as follows:

A homology with a given center and axis is characterized by the fact that it permutes with every collineation of G which permutes with the harmonic homology with the given center and axis.

In any automorphic mapping of G, the image of a homology is a homology and conversely, every homology is the image of some homology.

The product of two homologies with different centers and different axes is not a homology.

If t_1 and t_2 are harmonic homologies with a common center A, their images in any automorphism of G are harmonic homologies with a common center or a common axis, and dually.

Two homologies with a common center are carried by an automorphism into two homologies with a common center or a common axis and dually.

Any automorphism of G carries an elation into an elation, and conversely, every elation is an image of an elation.

The only subgroups of G which consist of homologies and elations alone are points, lines and their subgroups.

Let P be any point. If an automorphism T makes $a \rightarrow a^T$ for each element a of G then P^T, the group of all images of elements of P, is a point or a line. The dual is also true.

If an automorphism T maps one point into a point it maps every point into a point and every line into a line; if T maps one point into a line it maps every point into some line and every line into some point.

MAIN THEOREM: *Let G be the projective collineation group of a projective plane π. With each automorphism of G we can associate a unique collineation or correlation (not necessarily projective) of π, and, conversely, with each collineation or correlation of π we can associate a unique automorphism of G, the mapping being an isomorphism.*

If one introduces a coördinate system into the geometry it can easily be seen that the complete collineation and correlation group is isomorphic to the group of all semilinear transformations in the coördinate field.

The case of n-dimensional geometry follows exactly the same lines once it is established that an automorphism maps a harmonic homology into a harmonic homology.

The Euclidean Subgroup of G (Two-Dimensional Case).—The identification of the Euclidean group as the subgroup of G which keeps a line and an absolute involution in it invariant has been established many times on geometrical grounds. From our point of view it is interesting to see if the Euclidean and orthogonal subgroups of G could be obtained directly from our group-theoretic definition of G. This in itself would be a difficult problem but for the work of Thomsen.[1] In essence, Thomsen gives a group theoretic characterization of the orthogonal group and what we are able to accomplish is to show that Thomsen's axioms are implied by ours when a proper specialization is made.

* This paper is a summary of a thesis presented at the University of Toronto in June, 1942, for a Ph.D. degree. The work was done under the supervision of Prof. G. deB. Robinson, for whose many aids and suggestions my thanks are extended.

[1] Thomsen, *Math. Zeit.*, **34**, 668–720; **37**, 561–566.

[2] Hjelmslev, J., "Einleitung in die allgemeine Kongruenzlehre," *Det Kgl. Danske Videnskabernes Selskab. Math. Phys. Meddelser*, **8** (11) (1929) and **10** (1) (1929).

[3] Cf. Robinson, *Foundations of Geometry*, Toronto, 1940.

[4] This problem was first treated (in an entirely different manner) by Van der Waerden and Schreier, *Abh. Math. Sem. Hamb.*, **6**, 302–322 (1928).